

Insect succession and changes in the soil pH and electrical conductivity associated with decomposing pig carcasses on the Table Mountain National Park of the Western Cape Province of South Africa

By

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Abstract

The establishment of region-specific baseline data on carrion-associated entomofauna and changes in the chemistry of carrion-impacted soil is crucial in the application of forensic entomology and soil chemistry in post-mortem interval estimations. However, within the Western Cape Province of South Africa, and specifically, the Table Mountain region, no studies on carrion-associated entomofauna have been published. Also, no studies in South Africa have investigated how the changes in carrion-impacted soil chemistry can be used in post-mortem interval estimations.

Therefore, to establish baseline carrion entomofauna data, this study investigated the insect assemblages associated with decomposing neonate pig carcasses, alongside the impact of carcass decomposition on soil, carcass skin, and larval mass(es) chemistry, on a monthly temporal scale across the spring, summer, autumn and winter seasons. Secondly, in line with the quest for forensic relevance as evidenced by the observations from local forensic cases within the Province, this study examined the similarity in the collected carrion entomofauna, soil, carcass skin and larval mass(es) chemistry data generated from the neonate pig studies, with those generated from singly deployed adult pig carcasses across the winter and summer seasons. Thirdly, to further establish the forensic relevance of the entomofauna data generated from experimental studies, this study examined the insect species documented in forensic entomological reports of actual forensic cases within the Western Cape Province and compared the data therein with those generated from field studies using neonate and adult pig carcasses.

A total of twenty-four neonate pig carcasses (two for each monthly trial) were used to generate baseline data on carrion-associated entomofauna and changes in soil pH and electrical conductivity, carcass skin and larval mass(es) in each month across the spring, summer, autumn, and winter seasons, between August 2020 to September 2021. Similar data were collected over the winter and summer seasons respectively using two adult pig carcasses (one for each seasonal trial) between June 2022 to January 2023. The neonate pig carcasses decomposed twice as fast in the summer season when compared to the spring and autumn seasons, and four times faster than those deployed in the winter season. The adult pig carcass deployed in the summer season decomposed approximately eight times faster than that deployed in the winter season.

Generally, considerable differences were found in the insect species that successfully deposited their eggs/larvae on the neonate pig carcasses on a monthly and seasonal temporal scale. Certain insect species were observed to be seasonal in terms of their ability to deposit their eggs/larvae on vertebrate remains. The insect species that deposited their eggs/larvae on the neonate and adult pig carcasses were found to be similar across the summer and winter seasons. Likewise, there was a significant consistency between the insect species that colonized and deposited their eggs/larvae on the experimental models (neonate and adult pig carcasses) with those collected from decomposing human remains across the four seasons of the region. The presence and successful oviposition of the blow flies, *Calliphora croceipalpis* (Jaennicke, 1867), *Chrysomya megacephala* (Fabricius, 1794) and *Hemipyrellia fernandica* (Macquart, 1855) on decomposing pig and/or human remains as shown in this study, represents the first records of the presence of the adult and immature stages of these species on decomposing vertebrate remains in South Africa since the 1960s and 1980s, and further confirms their local forensic relevance. An unexpected finding of significant forensic importance was the creation of multiple skin lesions on the skin of the pig carcasses by *Crematogaster* cf. *liengmei*, which were exploited by blow flies as oviposition sites. These observations, amongst others, validate the need to conduct locality/region specific baseline decomposition and forensic entomological studies prior to the utilization of forensic taphonomic and entomological data in cause and time since death estimations.

In all the neonate and adult pig trials, the pH and electrical conductivity of the carcass soils differed significantly from the control soils. Furthermore, as decomposition progressed, carcass soil pH and electrical conductivity were observed to increase, albeit differently depending on the season. Generally, carcass skin pH in the neonate and adult pig carcasses increased gradually, exceeding the neutral pH mark on different days as decomposition progressed. The larval mass(es) pH was mostly above neutral, indicative of an alkaline environment, and likely a major contributor to the observed environmental and carcass skin pH changes. In summary, this study provides valuable baseline information on the activities of insects of forensic importance on decomposing vertebrate remains across several seasons for the Table Mountain region and Western Cape Province at large. Furthermore, it provides valuable baseline data on the changes in the chemistry of the soil and skin associated with decomposing carcasses alongside their potentials in minimum post-mortem interval estimations within the Western Cape Province of South Africa.

Conference Proceedings

1. **Adetimehin, A. D.,** Mole, C. G., Finaughty, D. A. and Heyns, M. (2023). Diversity, abundance, and successional patterns of insects on an adult pig carcass (*Sus scrofa domesticus* L.) in Cape Town, South Africa: preliminary investigation in the summer season. *A paper presented virtually at the Royal Entomological Society's Annual Conference, Ento23, University of Exeter, United Kingdom, September 5 – 7 2023.*
2. **Adetimehin, A. D.,** Mole, C. G., Finaughty, D. A. and Heyns, M. (2023). Incidental observation of bone modification by ants (*Crematogaster* cf. *liengmei*) in Cape Town, South Africa. *A paper presented at the XXVIII International Symposium on Morphological Sciences scheduled to take place at the University of Cape Town GSB Conference Centre, August 5 – 8 2023.*
3. **Adetimehin, A. D.,** Mole, C. G., Finaughty, D. A. and Heyns, M. (2023). Flowing with the stream: unidirectional dispersal of blow fly larvae following decomposition fluids from an adult pig carrion. *A paper presented virtually at the Royal Entomological Society Student Forum, Cardiff, United Kingdom, March 30 – 31, 2023.*
4. **Adetimehin, A. D.,** Mole, C. G., Finaughty, D. A. and Heyns, M. (2022). Diversity, abundance, and successional pattern of insects on neonate pigs (*Sus scrofa domesticus* L.) in the Western Cape Province of South Africa – a summer study. *A paper presented at the 18th Meeting of the European Association for Forensic Entomology, University of Alcalá, Madrid, Spain, June 16 – 17, 2022.*
5. **Adetimehin, A. D.,** Mole, C. G., Finaughty, D. A. and Heyns, M. (2022). Post-mortem excoriation and insect predation: activities of *Crematogaster* cf. *liengmei* (Hymenoptera: Formicidae) on an adult size pig carcass in the winter season of the Western Cape Province of South Africa. *A paper presented virtually at the 3rd Annual World Anatomical Society of Southern Africa World Anatomy Day Online Symposium, October 13, 2022.*
6. **Adetimehin, A. D.,** Mole, C. G., Finaughty, D. A. and Heyns, M. (2022). Decomposition in the Western Cape: Changes in soil pH and electrical conductivity in summer decomposition. *A paper presented virtually at the 49th Anatomical Society of Southern Africa Conference, April 19 – 21, 2022.*
7. **Adetimehin, A. D.,** Mole, C. G., Finaughty, D. and Heyns, M. (2021). Caught in the - act: impact of *Crematogaster* spp. (Hymenoptera: Formicidae) necrophagous behavior on neonate pigs (*Sus scrofa domesticus* L.) in the Western Cape Province of South

Africa. *A paper presented at the HUB-IBMS-Pathology Departmental Joint Research Day, Faculty of Health Sciences, University of Cape Town, South Africa, December 1 – 2, 2021.*

8. **Adetimehin, A. D.,** Finaughty, D. and Heyns, M. (2021). Changes in soil pH and electrical conductivity associated with decomposing neonate pigs (*Sus scrofa domesticus* L.) in the Western Cape Province of South Africa – a spring study. *A paper presented virtually at the 17th Meeting of the European Association for Forensic Entomology, University of Portsmouth, United Kingdom, June 9 – 11, 2022.*
9. **Adetimehin, A. D.,** Finaughty, D. and Heyns, M. (2021). Entomofauna and their pattern of succession on neonate pigs (*Sus scrofa domesticus* L.) in the Western Cape Province of South Africa – a spring study. *A paper presented virtually at the 22nd Congress of the Entomological Society of Southern Africa, Tshipise Forever Resort, South Africa, June 28 – 1 July 2021.*

Scientific Publications

- 1 **Adetimehin, A. D.,** Mole, C. G., Finaughty, D. A. and Heyns, M. (2023). Parasitic and predatory behavior of *Alysia manducator* (Hymenoptera: Braconidae) on blow fly larvae feeding on an adult pig carcass in the Western Cape Province of South Africa: preliminary observations and forensic implications. *International Journal of Legal Medicine*. 10.1007/s00414-023-03001-5.
- 2 **Adetimehin, A. D.,** Mole, C. G., Finaughty, D. A. and Heyns, M. (2023). Incidental observation of bone modification by *Crematogaster* cf. *liengmei* (Hymenoptera: Formicidae) in Cape Town, South Africa. *Forensic Science, Medicine and Pathology*. 10.1007/s12024-023-00714-2.
- 3 **Adetimehin, A. D.,** Mole, C. G., Finaughty, D. A. and Heyns, M. (2022). Caught in the act: impact of *Crematogaster* cf. *liengmei* (Hymenoptera: Formicidae) necrophagous behavior on neonate pigs (*Sus scrofa domesticus* L.) in the Western Cape Province of South Africa. *International Journal of Legal Medicine*. 10.1007/s00414-022-02835-9.

Dedication

This thesis is dedicated to:

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List of Abbreviations

ADD = Accumulated Degree Days

ADH = Accumulated Degree Hours

ADP = Adenosine Diphosphate

AEC = Animal Ethics Committee

AIC = Akaike's Information Criterion

ANOVA = Analysis of Variance

ATP = Adenosine Triphosphate

CapeFORTE = Cape Forensic Taphonomy and Entomology Laboratory

CDI = Cadaver Decomposition Island

CFK = Cape Floral Kingdom

DNA = Deoxyribonucleic Acid

GAM = Generalized Additive Model

GLM = Generalized Linear Model

HREC = Human Research Ethics Committee

HSD = Honest Significant Different

PIA = Period of Insect Activity

PMI = Post-mortem interval

PMI_{min} = Minimum post-mortem interval

RF = Rainfall

RH = Relative Humidity

SAPS = South African Police Service

SD = Standard Deviation

TBS = Total Body Score

TOC = Time Since Insect Colonization

UCT = University of Cape Town

USA = United States of America

Δ EC = Change in electrical conductivity

Δ pH = Change in pH

Chapter 1: Introduction and Literature Review

1.1. Definition and fundamentals of forensic entomology

In the broad sense, forensic entomology is the branch of forensic science that capitalizes on the biology and ecology of insects and other arthropods (e.g., mites, spiders, and crustaceans) for clarifying specific questions in medical, civil, and legal cases in the court of law (Catts and Goff, 1992; Byrd and Castner, 2010). According to Byrd and Castner (2010), this highly specialized branch of forensic science comprises three (3) broad categories namely, stored product pest forensic entomology, urban forensic entomology, and medico-legal forensic entomology.

Stored product pest forensic entomology focuses on the utilization of insects in resolving disputes relating to the presence of insects in food, food products and food production sites (Byrd and Castner, 2010). Urban forensic entomology entails the use of insects in finding solutions to civil actions concerning insect infestations in human-associated dwellings, buildings, and structures (Byrd and Castner, 2010). Medico-legal forensic entomology – sometimes referred to as medico-criminal entomology – deals with the use of insects in deducing the circumstances surrounding the demise of an individual, especially in cases suspected to be homicides, suicides, or unexplained deaths (Catts and Goff, 1992; Byrd and Castner, 2010). Above all, medico-legal forensic entomology is the principal area of interest in forensic entomology (Amendt *et al.*, 2007), and the central focus of this study. This category of forensic entomology further involves the utilization of insects in generating valuable information that can assist in resolving medico-legal cases including but not limited to the estimation of the minimum post-mortem interval (PMI_{min}) (Catts, 1992), presumption of season of death (Vanin and Huchet, 2017), detection of drugs and toxic compounds (Campobasso *et al.*, 2004), determination of the duration of neglect or abuse (Benecke *et al.*, 2004; Brundage and Byrd, 2016), detection of changes in cadaver location (Amendt *et al.*, 2011), wildlife poaching (Watson and Carlton, 2003), pre- or post-burial interval estimations (Singh *et al.*, 2016), and post-mortem submergence interval estimations (Wallace *et al.*, 2008). Amongst all of these, the estimation of the PMI_{min} is the center of attention of most forensic entomological studies (Amendt *et al.*, 2004).

In medico-legal related cases, the post-mortem interval (PMI) is usually one of the most important factors to determine during medico-legal investigations (Catts, 1992; Buchan and Anderson, 2001; Sharma *et al.*, 2015). This interval is defined as the period between the demise

of an individual and the discovery of their remains (Catts and Goff, 1992). The accurate estimation of the PMI is crucial not only in the reconstruction of the events leading to the death of an individual and the discovery of their remains, but also, in delimiting the pool of suspects who may have caused the death or are in close association with the crime or death scene (Li *et al.*, 2016; Keough *et al.*, 2017). Over the years, medical practitioners (including forensic pathologists) have been able to estimate the PMI of an individual shortly after death with the aid of several biological and chemical parameters (Goff, 2009; Li *et al.*, 2016; Gelderman *et al.*, 2019). However, most of these parameters are often less reliable after 72 hours post-mortem, and absent or inapplicable in dismembered and burnt bodies (Anderson and Vanlaerhoven, 1996; Vanin *et al.*, 2013; Vanin and Hutchet, 2017; Wang *et al.*, 2019). Consequently, attempts to use these parameters in the aforementioned circumstances can potentially lead to inaccuracies in the estimation of the PMI. Being the subject of extensive research and scientific scrutiny over the years, the analysis of the insect species associated with decomposing vertebrate remains (i.e., forensic entomology) has been found to be the most accurate and often reliable method in the estimation of the PMI in human remains beyond 72 hours post-mortem (Anderson and Vanlaerhoven, 1996; Buchan and Anderson, 2001).

1.2. Brief history of forensic entomology

The history of forensic entomology globally has been well reviewed by several authors (Greenberg, 1991; Benecke, 2001; Greenberg and Kunich, 2002; Amendt *et al.*, 2004; Benecke, 2008; Byrd and Castner, 2010; Gennard, 2012). However, provided below is a summary.

The first recorded event in which insects were used in criminal investigations dates to the thirteenth century in an agricultural village in China. A Chinese expert in criminal cases (Sung Tz'u) was reported to have used insects (blow flies) to identify a sickle used to kill a farm worker. In the mid-18th century, the first application of the knowledge of forensic entomology in the estimation of the PMI in the court of law was reportedly carried out by a French doctor named Louis Bergeret. Bergeret reportedly investigated a case involving the discovery of the remains of an infant in a confined fireplace. Following the analysis of the insect species found in association with the remains, Bergeret was documented to have identified the insects to be a flesh fly and moth. Bergeret provided an estimate of the PMI which subsequently led to the exoneration of the recent occupants of the building. However, it was noted that his estimate of the PMI based on the life cycle of both insects was incorrect and that the time since death of the infant might have been shorter.

In 1878, during the autopsy of a mummified newborn child, Paul Brouardel observed several larvae of butterflies and individuals of mites in and around the remains of the child. Following his observations, he was documented to have consulted two individuals namely Perier and Pierre Mégnin both of whom assisted in the identification of the butterfly larvae and mites respectively. Based on the analysis of the butterfly larvae and the state of decomposition of the remains, Perier revealed that the newborn must have died 6 – 7 months before the remains were autopsied. However, based on the sex and numerical abundance of mites found in several parts of the remains, Pierre Mégnin was able to conservatively estimate the duration of corpse abandonment to at least 5 months, and possibly, up to 7 – 8 months.

In 1894, the book published by Pierre Mégnin, entitled “*La Fauna des cadavers: Application de l’entomologie a la medicine legale*” reportedly brought the science of forensic entomology and its application in medico-legal investigations to the limelight. In 1935, the analysis and estimation of the ages of third instar larvae associated with the dismembered body parts of two human remains found in a river close to Edinburgh, Scotland, provided valuable information relating to the time since deposition of the remains and this proved to be sufficient. With a major focus on case work, the science of forensic entomology was principally practiced by Leclercq and Nuorteva between the 1960s and 1980s in central Europe, and much of this, alongside additional research, paved the way for the ongoing studies and application of forensic entomology in medico-criminal investigation seen in recent times.

1.3. Current state of the training of forensic entomologists

About a decade ago, Magni *et al.* (2013) conducted a survey that provided valuable insights on the status of forensic entomology practitioners associated with four forensic entomology specialist organizations. The results from the study revealed that a significant disparity existed in the educational status of practicing forensic entomologists. According to the authors, out of 69 respondents, approximately 80% indicated that they had acquired higher levels of formal education such as Masters and Doctorates while the remaining respondents had obtained either Bachelors degrees (11%), had no qualifications (3%), or had obtained some other type of qualification (6%).

Secondly, the authors further highlighted the lack of consistency in the accreditation and certification processes of forensic entomologists across several countries. While most of the respondents acknowledged the existence of accreditation processes prior to becoming a forensic entomology practitioner in their countries, a worrisome issue was the lack of

awareness in some practicing forensic entomologists with regards to the existence of accreditation procedures within the same country. Thirdly, based on the responses in their survey, the authors revealed that most forensic entomologists drafted entomological case reports, while some seldom did. An extra layer of complication to this issue as documented by the authors was the lack of a standardized case reporting format globally. All these inconsistencies further highlight the need for validation of forensic entomology as a scientific method especially where the estimation of the PMI in the courts of law is concerned.

1.4. Forensic entomological research in South Africa

The history of forensic entomology in South Africa has been thoroughly documented (see Williams and Villet, 2006a; Tembe and Mukaratirwa, 2020). Detailed below is an overview of forensic entomology related studies conducted across the Provinces in South Africa.

The first reported and published studies categorically focusing on forensic entomology were those of Andre Prins (Williams and Villet, 2006a). In 1980, Prins investigated the insect species associated with different decomposing vertebrate carrion in the Eastern and Western Cape Provinces. Following Prins' pioneering research and publications bordering around the ecology, morphology, and developmental rates of blow flies in the 1980s, several research studies in various academic institutions in the different Provinces within the country began to explore diverse areas of forensic entomology (Williams and Villet, 2006a).

A large portion of research has been conducted in the Free State Province. Kelly (2006) investigated the diversity, abundance and successional patterns of insects associated with decomposing pig carcasses across the four seasons of the region. Various scenarios were investigated including the effect of clothing and presence of injury and this culminated in three publications (Kelly *et al.*, 2008, 2009, 2011). Kolver (2003, 2009) investigated the diversity, abundance and successional patterns of insects associated with decomposing pig carcasses which were hung, burnt and unburnt across the four seasons. Brink (2009) generated key diagnostic features useful in the identification of the immature stages of locally available forensically important insect species belonging to the families Calliphoridae and Sarcophagidae. Botham (2016) and van der Merwe (2016) investigated the successional patterns of insects associated with buried and unburied pig carcasses in the winter and summer seasons. Recently, van der Westhuizen (2021) investigated the changes in the development and gene expression of *Chrysomya albiceps* (Weidemann, 1819) during the intra-puparial developmental stages.

In the Eastern Cape Province, a wide range of forensic entomological studies have been conducted. Richards (2007) investigated the effects of temperature on the development, behavior, and geographical distribution of blow flies in the forensic context. The findings from the study culminated in four scientific publications (Richards *et al.*, 2008, 2009a, b, 2013). Furthermore, studies on entomotoxicology, nocturnal oviposition of blow flies, systematics, taxonomy, identification, development, and geographical distribution, of insects (flies and beetles) of forensic importance have been undertaken (Musvasva *et al.*, 2001; Lunt, 2002, da Silva and Villet, 2006; Williams and Villet, 2006a, b; Midgley and Villet, 2009a, b; Williams *et al.*, 2014, 2017; Williams and Villet, 2013, 2014, 2019; Ridgeway *et al.*, 2014; Kotzé *et al.*, 2015, 2016; Daniel *et al.*, 2017; Villet *et al.*, 2017; Williams *et al.*, 2021).

In the Gauteng Province, Gilbert (2014) investigated the blow fly species associated with decomposing pig carcasses in the summer and winter seasons. In addition, the author examined the cephaloskeleton and spiracles of blow fly larvae in a bid to establish characteristic morphological features that can assist in insect species identification. Parry *et al.* (2016) investigated the variation in the assemblages of carrion-associated Dipterous flies across different seasons, habitats, and localities. Hill (2019) investigated the insect species associated with decomposing human remains during and after autopsy procedures at the Johannesburg Pathological Service Mortuary. Hill further provided a simulated model on the influence of temperature on cadaver-associated Dipterous insects from the scene of discovery to refrigeration and autopsy (Hill *et al.*, 2020).

In the KwaZulu-Natal Province, studies focusing on the molecular identification, diversity, abundance, and successional patterns of insects (flies and beetles) on small and large decomposing vertebrate (i.e., rats, pig, and sheep) remains across multiple seasons of the region have been conducted (Meskin, 1986; Tembe and Mukaratirwa, 2021; Tembe *et al.*, 2021a, b).

Across the Mpumalanga and Limpopo Provinces, studies have been conducted on the effect of scavenger dismemberment on insect succession alongside the diversity, abundance and successional patterns of insect species associated with decomposing pig, and impala carcasses (Braack, 1981, 1984, 1986, 1987; Ellison, 1990) in various seasons.

In the Western Cape Province, Sanyanga (2016) investigated the effect of amitriptyline, an anti-depressant medication on the growth of blow fly larvae. Similarly, Visser (2016) examined the effect of methylphenidate hydrochloride on the developmental process of *Chrysomya chloropyga* (Weidemann, 1818). Cooke *et al.* (2018) utilized deoxyribonucleic acid

(DNA) barcodes in the identification of four blow fly species from one locality within the Province. Kulenkampff (2019) expanded on Cooke's study by generating additional barcodes for seven forensically important blow fly species from six localities within the province. Finaughty (2019) established the first baseline data on soft-tissue decomposition and necrophagic arthropod occurrence and succession in the summer and winter seasons of the region in almost three decades. Alberts (2021) analyzed the external morphological characteristics of *Lucilia sericata* (Meigen, 1826) pupae for the purpose of age estimation. Adetimehin *et al.* (2022) revealed interesting observations relating to the necrophagic, and predatory behavior of the ant species, *Crematogaster cf. liengmei* on decomposing neonate pig carcasses across the four seasons of the region. Adetimehin *et al.* (2023a) provided valuable information on the forensic implications of the predatory and parasitic behaviors of the Braconid wasp, *Alysia manducator* (Panzer, 1799) on blow fly larvae feeding on pig carrion.

Evidently, forensic entomology is a growing research area in South Africa. Research encapsulates some but not all geographic regions in the country as detailed above. Thus, further research focused on generating local and region-specific baseline data on the development, diversity, and successional patterns of forensically relevant insect species is crucial (Byrd and Castner, 2010). Also, necrophagic insects have a close relationship with carrion given their adaptation to exploit this resource niche. Hence, understanding their biology requires an intimate knowledge of their substrate of choice.

1.5. Death and Decomposition

After the demise of an individual, the body begins to undergo a series of complex chemical, biological and physical processes collectively known as decomposition (Anderson and Vanlaerhoven, 1996). In the practical sense, decomposition is a continuous process, and the transition from one decompositional event to another is not distinct and the changes have no fixed duration (Amendt *et al.*, 2010; Gennard, 2012). Nonetheless, for convenience, and depending on the geographical location and animal model used, several authors have sought to delineate the process of decomposition into several stages ranging from one to as many as nine (Amendt *et al.*, 2010). In Costa Rica, only one stage of decomposition was noted by Cornaby (1974) using toad and lizard carcasses. In rural Tennessee, United States of America (USA), Reed (1958) categorized the stages of decomposition of dog carcasses into four (4) stages namely: fresh, early decomposition, advanced decomposition and skeletonization. Payne (1965), who conducted a summer study on the decomposition of neonate pig carcasses in South Carolina, USA categorized the decomposition of pig carcasses protected from arthropods into

five viz. fresh, bloating and decomposition, flaccidity and dehydration, mummy, and desiccation and dehydration. Furthermore, the author categorized the decomposition of neonate pig carcasses exposed to arthropods into six: fresh, bloated, active decay, advanced decay, dry stage and remains. Rodriguez and Bass's (1983) categorization includes fresh, bloated, decay and dry, while Galloway *et al.*'s (1989) categorization includes fresh, early decomposition, advanced decomposition and skeletonization. Amendt *et al.* (2010) noted that Ménégnin identified nine stages of decomposition in human cadavers.

In an attempt to quantitatively describe the stages of decomposition of a human cadaver, Megyesi *et al.* (2005) developed the Total Body Scoring (TBS) system by retrospectively analyzing photographs associated with sixty-eight human forensic cases with known PMI. The TBS system was the first of its kind and allocates body scores of 1 to 13, 1 to 12 and 1 to 10 respectively to each of the three distinct regions of the body (- head and neck, abdomen, and limbs), based on the physical changes the body undergoes. The sum of the body scores generated from the three body regions produces the TBS, that enables one to delineate the decomposition process into four stages namely: fresh, early decomposition, advanced decomposition and skeletonization. Given that experimental studies on vertebrate decomposition utilize animal models (e.g., pigs and rabbits), Keough *et al.* (2017) revised the human TBS scoring system and established a TBS scoring system for pig carcasses. Using this, they were able to categorize the decomposition of pig carcasses into the four stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization).

Despite the categorization of the process of decomposition into varying stages by different authors, a series of early and late changes occur in the body post-mortem which invariably results in the conversion of a fully fleshed body into skeletal materials (Goff, 2009; Amendt *et al.*, 2010). One of the early changes is the termination of blood flow in the body following the cessation of the beating of the heart. Due to gravity, the blood starts to settle and accumulate at the lower extremities of the body (relative to gravitational influence). This blood accumulation leads to the discoloration of selected body regions especially in the areas around the lower extremities usually in contact with the floor or any stable physical surface. This process is referred to as *livor mortis*, lividity, or post-mortem hypostasis (Amendt *et al.*, 2010). Within 1 hour post-mortem, physical observation of the signs of lividity are often noticeable, with full presentation noticeable within 3 – 4 hours. As the body continues to decompose, chemical changes in the myofibrils of the muscle tissues linked to the conversion of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and lactic acid, alongside the resultant

decline in cellular pH levels, brings about the stiffening of the muscles of the face and other parts of the body, such as the limbs (Amendt *et al.*, 2010). This characteristic stiffening or rigidity of the muscles is known as *rigor mortis* and it begins within 2 – 6 hours post-mortem (Amendt *et al.*, 2010). Changes in the body temperature also occurs at the onset of death and is connected to the cessation of temperature regulation. At this stage, the body temperature becomes equivalent to that of the immediate environment (Amendt *et al.*, 2010). This most commonly manifests as a drop in temperature and is known as *algor mortis*, generally occurring within 18 – 20 hours post-mortem.

Midway through the decomposition process, if the eyes are open, a change in the coloration of the exposed cornea becomes noticeable. Also, changes in the appearance and coloration of the blood vessels from red to green become evident and this is as a result of the proliferation of gases (mostly hydrogen sulphide) and the formation of sulfhemoglobin through the reaction of hydrogen sulphide with hemoglobin in the blood. Furthermore, because of the migration and spread of anaerobic bacteria, marbling occurs, which is characterized by a greenish-purple discoloration of the subcutaneous vessels specifically around the abdominal region and limbs (Amendt *et al.*, 2010). In certain environmental conditions (e.g., wet, or moist habitats), the separation of the epidermis from the dermis occurs and this is often linked to the proliferation of hydrolytic enzymes. As a result, the skin surrounding the hands and feet pulls away partially or wholly from the underlying dermal layer (Amendt *et al.*, 2010). This condition is known as skin slippage or glove formation (Amendt *et al.*, 2010).

As decomposition progresses into the late stages, an increase in internal body pressure occurs and this results in the discharge of decomposition fluids and gases in the body into the immediate environment via body openings such as the mouth, ears, nose, and anus (Carter *et al.*, 2007). This subsequently results in the collapse of the inflated abdominal region. Thereafter, apneumones (volatile organic compounds) present in the liquid and gaseous discharge from the body, attract and stimulate the activities of a wide variety of micro-organisms (aerobic and anaerobic bacteria, fungi), insects (flies, beetles, ants, and wasps) and vertebrate scavengers (rodents, canines, felines, and avians among others) (Joseph *et al.*, 2011; Verheggen *et al.*, 2017).

Importantly, deviation from the above-mentioned changes may occur to the body under certain environments (e.g., arid environments) or conditions (e.g., extreme heat or cold). Under these conditions, a vertebrate body may progress into a state of long-term preservation called

mummification. In this state, the outer layer of the body (skin) becomes desiccated owing to reduced bacterial and insect activity. In addition, the skin and other desiccated tissues become leathery in appearance, whilst contracting tightly and forming a shell-like structure over the underlying bones and greasy substances (Amendt *et al.*, 2010; Brooks, 2016; Gelderman *et al.*, 2019; Finaughty and Morris, 2019). Equally important, under cold and moist conditions, the conversion of triglycerides into glycerine and fatty acids via hydrolysis brings about the decomposition of the adipose tissues in the body. This subsequently results in the formation of a soft grey-white substance known as corpse wax or adipocere (Schotsmans *et al.*, 2011; Ermida *et al.*, 2022). Over time, this substance becomes hard and brittle and, as a result, leads to a delay in the process of decomposition through the formation of a barrier layer on the body (Schotsmans *et al.*, 2011; Ermida *et al.*, 2022).

1.5.1. Factors affecting decomposition

The process of decomposition of vertebrate remains has been documented to be influenced by numerous factors. These factors can be categorized into intrinsic and extrinsic factors (Campobasso *et al.*, 2001; and Gelderman *et al.*, 2019).

Intrinsic factors affecting the process of decomposition are those that are attributable to the remains themselves (Campobasso *et al.*, 2001; Gelderman *et al.*, 2019), including (1) cause of death, (2) age at death, (3) body size, weight, and constitution, and (4) integrity of the remains (e.g., presence of trauma) (Campobasso *et al.*, 2001; Zhou and Byard, 2011; Gelderman *et al.*, 2019). The cause of death plays a key role in determining the rate of decomposition of vertebrate remains. For instance, in deaths associated with infectious diseases, the rate of decomposition is usually accelerated due to an increase in bacterial abundance and diversity in the blood and organs, alongside the potential increase in body temperature post-mortem (Campobasso *et al.*, 2001; Zhou and Byard, 2011). In contrast, deaths due to exsanguination may decelerate the rate or process of decomposition by virtue of the loss of blood that serves as a medium for the transport and spread of putrefactive bacteria (Zhou and Byard, 2011). Furthermore, substantial blood loss can lead to a deficiency in hemoglobin and other blood-associated proteins that serve as useful energy sources to these putrefactive bacteria, thus impacting the decomposition process (Zhou and Byard, 2011).

With regard to the age at death, it has been reported that decomposition in fetuses and newborn infants proceeds at a slower rate when compared to adult individuals (Campobasso *et al.*, 2001). Body size may play an additional role in the rate of decomposition, however there

is ongoing debate regarding whether an increase in body size increases the rate of decomposition (Roberts *et al.*, 2017). Some authors have shown that the rate of decomposition of large sized carcasses proceeded at a slower rate than that of small sized carcasses (Komar and Beattie, 1998; Spicka *et al.*, 2011; Sutherland *et al.*, 2013). In contrast, other authors have shown that decomposition proceeds at a faster rate in a large size carcass in comparison to a small size carcass (Mann *et al.*, 1990; Hewadikaram and Golf, 1991) or that body mass has no substantial impact on the decomposition rate of vertebrate remains (Roberts *et al.*, 2017). Body size is believed to be confounded by the presence or absence of insects, with smaller sized carcasses decomposing faster when compared to large carcasses (Simmons *et al.*, 2010). When insects are excluded, however, the carcass size exerts no influence on the rate of decomposition (Simmons *et al.*, 2010).

Reports on the effects of trauma or wounds on the rate of decomposition are variable. In general, trauma has been widely suggested by several authors to speed up the rate of decomposition of vertebrate remains by providing insects and other scavengers easier access to the soft tissues of the body and additional sites for bacteria infestation, and insect reproduction (Campobasso *et al.*, 2001; Byrd and Castner, 2010; Munro *et al.*, 2019). Experimental studies by several authors have shown that peri- and/or post-mortem penetrative trauma alone does not bring about any quantifiable increase in the rate of decomposition (Cross and Simmons, 2010; Kelly, 2006; Smith, 2014; and Munro *et al.*, 2019). However, these authors have noted considerable alteration in the pattern of decomposition attributable to the presence of trauma. With regard to insects' attractiveness to the area in or around peri- and/or post-mortem penetrative trauma, the results from the studies conducted by several authors revealed that trauma sites were not preferentially used for reproduction by adult female flies and that female flies still oviposited in the natural body orifices (mouth, eyes, and ears) and other areas of the body (Cross and Simmons, 2010; Kelly *et al.*, 2011; Charabidze *et al.*, 2015; Rivers *et al.*, 2023).

The extrinsic factors that influence the rate and pattern of decomposition of vertebrate remains can be categorized into two broad categories which include the abiotic and biotic factors. The biotic factors include microorganisms (e.g., fungi and bacteria), insects and vertebrate scavengers. The abiotic factors include ambient temperature, air humidity, rainfall, and others related to the handling or treatment of the remains (Amendt *et al.*, 2010; Rodriguez and Bass, 1983; Mann *et al.*, 1990; Campobasso *et al.*, 2001; Gelderman *et al.*, 2019).

The abiotic factors are non-living extrinsic factors that are involved in the process of decomposition. Ambient temperature is arguably the most important abiotic factor that affects the rate and pattern of decomposition of vertebrate remains (Rodriguez and Bass, 1983; Mann *et al.*, 1990; Vass *et al.*, 1992; Campobasso *et al.*, 2001; Iancu *et al.*, 2018; Charabidze and Hedouin, 2019). This is due to its influence on the biological and chemical processes associated with the decomposition of vertebrate remains (Verheggen *et al.*, 2017). Under low temperatures (i.e., cold, or freezing temperatures), the activities and proliferation of the putrefactive bacteria, alongside the activities, reproduction, and development of insects, associated with the decomposition process are usually impeded or terminated (Rodriguez and Bass, 1983; Amendt *et al.*, 2010). This, in turn, leads to a reduction or cessation in the decomposition process of the vertebrate remains (Rodriguez and Bass, 1983; Mann *et al.*, 1990; Amendt *et al.*, 2010). Conversely, under moderately high temperatures (between 25 – 35°C), the activities and proliferation of putrefactive bacteria and endogenous enzymes associated with decomposition are usually accelerated, therefore leading to an increase in the putrefaction of the body tissues (Spennemann and Franke, 1995; Campobasso *et al.*, 2001; Aufderheide, 2011). In addition, the activities, diversity, reproduction, and development of insects associated with decomposing vertebrate remains are usually increased under these conditions (i.e., moderately high temperatures), and as such, the rate of decomposition is accelerated (Rodriguez and Bass, 1983; Campobasso *et al.*, 2001).

In environments with extremely elevated temperatures, the activities of putrefactive bacteria and carrion-associated insects may be decreased or terminated, thus leading to the transition of the vertebrate body to mummification (Amendt *et al.*, 2010). It is noteworthy that the ambient temperature alone does not contribute entirely to the biological and chemical processes that occur during decomposition. Although temperature influences other abiotic factors such as the speed of the wind and air humidity, all these factors (including temperature) exert a combined influence on the rate of decomposition (Finaughty, 2019). In dry and windy environments for instance, vertebrate remains lose moisture rapidly, and this leads to a reduction in the proliferation of putrefactive bacteria, and further triggers the onset of mummification (Campobasso *et al.*, 2001). Under humid conditions, the body tissues retain moisture, and as a result, the rate of decomposition is slowed down (Campobasso *et al.*, 2001). In addition, the decrease in the chances of vertebrate carcass colonization by blow flies, which is a resultant effect associated with an increase in humidity (George *et al.*, 2013), may slow down the process of decomposition.

The effect of rainfall on the rate of decomposition of vertebrate remains appears to be variable and has not independently received sufficient attention in the forensic literature. However, some authors – as part of their studies in forensic entomology – have attempted to explain how rainfall influences the process associated with the decomposition of vertebrate remains. According to Archer (2004), higher temperatures and rainfall were observed to increase the rate of body mass loss and further decrease the duration of the decomposition stages of exposed neonate pig carcasses. Archer (2004) suggested that rainfall can contribute to the mechanical disintegration of the flesh, thus creating potential entry sites for insects and other microbes. In addition, rainfall tends to keep the area around the carcass moist, and this prevents the carcass from drying out, and in turn, facilitates maggot and bacteria activities (Archer, 2004). Rainfall may also slow down the rate of decomposition of vertebrate remains by suppressing or disrupting the dissipation of the volatile organic chemicals from the decomposition environment, preventing insect access, and by washing away or drowning fly eggs and larvae associated with the remains (Anderson and Vanlaerhoven, 1996; Amendt *et al.*, 2010; George *et al.*, 2013; McLeod, 2015; Brownlow *et al.*, 2023). Mahat *et al.* (2009) revealed that rainfall delayed the oviposition of blow flies on vertebrate remains by 1 to 2 days and also prolonged the duration of pupariation by 1 to 3 days.

Other abiotic factors such as the pH of the surrounding soil environment, presence of an external body coverage (e.g., clothing, wrapping), treatment (e.g., embalming, smoking, pickling) and depositional environment (above ground, hanging, buried, under shade, vehicular confinement, trashcan, suitcase and drum) of the remains post-mortem may indirectly affect the rate and pattern of decomposition of vertebrate remains by limiting or excluding the access of several biological and chemical factors (e.g., insects, moisture, and temperature) associated with the decomposition process (Galloway *et al.*, 1989; Mann *et al.*, 1990; Goff, 2009; Amendt *et al.*, 2010; Voss *et al.*, 2011; Lynch-Aird *et al.*, 2015; Finaughty, 2019; Lutz *et al.*, 2019; Gelderman *et al.*, 2019; Jarmusz *et al.*, 2020; Malainey and Anderson, 2020).

The biotic factors are simply the living organisms associated with the decomposition of vertebrate remains in a specific environment. According to Goff (2009), they can be categorized into four (4) categories. These include (a) bacteria, (b) fungi/mold, (c) vertebrate scavengers and (d) insects and acari. The role of bacteria in the decomposition of vertebrate remains in relation to the scope of this study has been previously described above and will not be described further. With regards to fungi and mold, there is a paucity of experts trained in mycology and its application in the forensic context (Tranchida *et al.*, 2021). Thus, studies

describing their contribution in the process of decomposition are scarce, and their specific role in the decomposition process is not well known. Although, Goff (2009) stated that the colonization of the corpse by mold and fungal spores result in the formation of “significant mats” on the body after death. Above all, these four categories of living organisms exert a synergistic impact on the overall process of decomposition (Goff, 2009).

A wide variety of vertebrate scavengers have been documented to be attracted to decomposing human and animal remains in various parts of the world (Byrd and Castner, 2010; King *et al.*, 2016; Verheggen *et al.*, 2017; Spies *et al.*, 2018; Finaughty, 2019; Dibner *et al.*, 2019; Keyes *et al.*, 2021; Sawyer *et al.*, 2022; Flint *et al.*, 2022). The results from these studies have shown that vertebrate scavengers can cause significant disarticulation and scattering of several parts/appendages of the carrion which in turn can complicate their recovery process and process of carrion decomposition. Furthermore, the obligatory and facultative feeding activities of some of these scavengers on the vertebrate carrion and carrion-associated insects (e.g., larvae) alter PMI estimations using insect evidence, and create post-mortem artefacts on bones and tissues which can be misinterpreted as antemortem or peri-mortem excoriations during forensic investigations (Byrd and Castner, 2010; King *et al.*, 2016; Verheggen *et al.*, 2017; Spies *et al.*, 2018; Finaughty, 2019; Dibner *et al.*, 2019; Keyes *et al.*, 2021; Sawyer *et al.*, 2022; Flint *et al.*, 2022). Equally important, vertebrate scavengers can feed on a vertebrate carrion, and subsequently create oviposition sites attractive and suitable for insects to lay their eggs and have direct access to the soft tissues of the body (Flint *et al.*, 2022).

In the absence of vertebrate scavengers and physical barriers, insects are by far the most important biotic agents that contribute to the process of decomposition of vertebrate remains (Mann *et al.*, 1990). These biotic agents have been categorized into four distinct groups based on their feeding habits on, in and around decomposing vertebrate remains. According to Smith (1986), these include (1) necrophages, which feed on the vertebrate remains (2) predators and parasites, which prey on and parasitize other insects associated with the body (3) omnivores, which feed on the vertebrate remains and its associated insects and (4) adventives, which utilizes the vertebrate remains as part of their habitat. Amongst these four categories, the necrophages, and predators and parasites, are the most important drivers of the process of decomposition (Finaughty, 2019).

Dipterous insects belonging to the families Calliphoridae (blow flies), Muscidae (house flies), and Sarcophagidae (flesh flies) are usually one of the first colonizers of decomposing

vertebrate remains, arriving within minutes, hours, or days after death (Byrd and Castner, 2010; Verheggen *et al.*, 2017). These insects are attracted to vertebrate remains by the volatile organic compounds associated with the gaseous and liquid discharges from the body. Following their arrival, gravid female individuals of these three insect families oviposit/larviposit in or close to natural body openings (e.g., ocular, nasal, or oral cavities), between the limbs or underneath the vertebrate carrion after which the emerging larvae start feeding on the soft tissues (Byrd and Castner, 2010; Verheggen *et al.*, 2017). In the late stages of decomposition, members of other insect families such as Piophilidae (cheese skippers), Sepsidae (black scavenger flies) and Scathophagidae (dung flies) arrive on the vertebrate remains and are attracted to the exposed greasy intestinal contents and dry proteinaceous food materials such as carrion, bones, skin, and fur (Byrd and Castner, 2010). Members of the family Phoridae (scuttle flies) appear to prefer carcasses buried or in confined environments (Disney, 2008; Reibe and Madea, 2010; Zuha *et al.*, 2017).

During the late phase of early decomposition and advanced decomposition stages, flies are accompanied by some beetles. Members of the beetle families such as Histeridae (clown beetles), Silphidae (carrion beetles), Scarabaeidae (scarab beetles) and Staphylinidae (rove beetles) are often found underneath a carcass, especially within the underlying soil and around the greasy intestinal substances. They prey on the immature stages of flies (Meskin, 1986; Braack, 1987; Byrd and Castner, 2010). In some instances, members of the families Histeridae and Staphylinidae arrive on the carcass shortly after the deposition of fly eggs or larvae, following which they commence their predatory behavior (Byrd and Castner, 2010). As the carcass begin to approach the skeletonization stage, members of other beetle families such as Dermestidae (skin beetles), Trogidae (hide beetles), and Cleridae (checkered beetles) arrive on the carrion, feeding on the dried tissues and skin of the carrion. This brings about the onset of skeletonization. Above all, considerable geographical variations exist on the successional patterns of insects on decomposing vertebrate remains (Byrd and Castner, 2010).

Other insect groups have also been documented to influence the process of carrion decomposition and subsequent vertebrate and invertebrate scavenger colonization of the carrion (Eubanks *et al.*, 2019; Spencer *et al.*, 2020; Cairncross *et al.*, 2022; Adetimehin *et al.*, 2022). Insects such as ants and wasps have been reported to exert necrophagous, predatory and parasitic behaviors on the flesh of vertebrate remains and the attendant insect developmental stages (eggs, larvae, and pupae) and assemblages in various parts of the world (Heo *et al.*, 2009; Moretti *et al.*, 2011; Cammack *et al.*, 2010; Frederickx *et al.*, 2013; Eubanks *et al.*, 2019;

Spencer *et al.*, 2020; Cairncross *et al.*, 2022; Adetimehin *et al.*, 2022, 2023a; Baumjohann and Benecke, 2022). These predatory, parasitic, and necrophagous behaviors have been suggested to influence the estimation of the PMI_{min} and potentially, could misinform forensic investigators during cause-of-death investigations (Moretti *et al.*, 2011; Rivers, 2016; Eubanks *et al.*, 2019; Somavilla *et al.*, 2019; Baumjohann and Benecke, 2022; Adetimehin *et al.*, 2022, 2023a). As insects play a vital role in PMI_{min} estimations, it is worth mentioning that factors such as season, geographical location, temperature, humidity, rainfall, clothing, injuries, burning, chemicals, drugs, concealment, and presence of vertebrate scavengers can influence their diversity, abundance, and successional patterns on decomposing vertebrate remains (Byrd and Castner, 2010; Vanin and Huchet, 2017). Due to the variation in insect assemblages across different geographical areas, regions, and seasons, it is important to first establish local, region and season-specific baseline data on the diversity, abundance, and successional patterns of carrion-associated insects prior to the utilization of entomological evidence in actual forensic cases within the region (Byrd and Castner, 2010; Cruise *et al.*, 2018; Díaz-Aranda *et al.*, 2018).

1.6. Physico-chemical changes in carrion-impacted soils

In most terrestrial ecological systems, the bulk of decomposing materials are from plants and fecal matter (Carter *et al.*, 2007). Owing to this, substantial attention has been given to the decomposition of plants and other plant-based products such as leaf litter, stems, seeds, and root exudates (Carter *et al.*, 2007; Permenter and MacMahon, 2009). Vertebrate carrion on the other hand, alongside their decompositional impact on soil processes within the terrestrial ecosystem, have not received similar amounts of attention despite their occurrence and ecological importance in terrestrial environments (Carter *et al.*, 2007; Benninger *et al.*, 2008; Permenter and MacMahon, 2009; Ololajulo, 2017; Baliso *et al.*, 2019, 2023). Vertebrate remains are ephemeral rich energy resources that play a key role in the cycling of nutrients in all ecological systems (Carter *et al.*, 2007; Barton *et al.*, 2013; Olakanye *et al.*, 2017; Woelber-Kastner *et al.*, 2021).

The processes associated with the decomposition of vertebrate remains (i.e., microbial, insect, and vertebrate scavenger activities) result in the release of a significant amount of organic materials into the surrounding environment (Towne, 2000; Carter *et al.*, 2007; Benninger *et al.*, 2008; Permenter and Macmahon, 2009; Byrd and Castner, 2010; Aitkenhead-Peterson *et al.*, 2012; Szelecz *et al.*, 2018a, b; Singh *et al.*, 2018; Barton *et al.*, 2020; Probst *et al.*, 2020; Woelber-Kastner *et al.*, 2021). Furthermore, these processes have been reported to bring about the formation of a nutrient-rich region underneath and around the carcass referred

to as the Cadaver Decomposition Island (CDI) (Carter *et al.*, 2007; Benninger *et al.*, 2008; Barton *et al.*, 2013; Singh *et al.*, 2018, 2023). Within the CDI, nutrients are deposited that are made up of carbon-, nitrogen- and phosphorus-based molecules which are majorly the by-products of the carbohydrates, proteins and lipids stored in vertebrates (Carter *et al.*, 2007; Benninger *et al.*, 2008; Barton *et al.*, 2013; Szelecz *et al.*, 2018a). The formation of the CDI has been documented to have substantial and persistent impacts on the underlying and surrounding soil chemistry, microbial, plant and insect communities (Towne, 2000; Carter *et al.*, 2007; Wilson *et al.*, 2007; Permenter and MacMahon, 2009; Barton *et al.*, 2013; Woelber-Kastner *et al.*, 2021; DeBruyn *et al.*, 2021). Hence, understanding these effects is crucial not only to forensic taphonomy and entomology, but also, the development of a wide range of techniques in the estimation of the PMImin (Szelecz *et al.*, 2018a).

Within terrestrial environments, the impact of cadaver decomposition on soil microbial activity and physico-chemical properties (e.g., pH, electrical conductivity, moisture content, nutrient concentration, and elemental composition) have been investigated by several authors (Towne, 2000; Carter *et al.*, 2007; Benninger *et al.*, 2008; Permenter and Macmahon, 2009; Aitkenhead-Peterson *et al.*, 2012; Cobaugh *et al.*, 2015; Fancher *et al.*, 2017; Singh *et al.*, 2018; Szelecz *et al.*, 2018a, b; Barton *et al.*, 2020; DeBruyn *et al.*, 2021; Woelber-Kastner *et al.*, 2021; Taylor *et al.*, 2023). Although certain trends are evident in these studies, there appears to be no clear consensus with respect to how some selected soil physico-chemical properties change over time as carrion decomposition progresses. A substantial degree of variability exists in the changes in pH of carrion-impacted soils over time. Some authors have demonstrated that carrion decomposition leads to an increase in the pH of the underlying soils associated with both exposed and buried vertebrate remains (Benninger *et al.*, 2008; Permenter and MacMahon, 2009; Bachmann and Simmons, 2010; Meyer *et al.*, 2013; Olakanye *et al.*, 2017; Szelecz *et al.*, 2018a; DeBryun *et al.*, 2021; Woelber-Kastner *et al.*, 2021; Rai *et al.*, 2022). Other authors have shown that carrion decomposition brings about a decrease (Towne, 2000; Aitkenhead-Peterson *et al.*, 2012; Perrault and Forbes, 2016; Taylor *et al.*, 2023), and at times, no substantial changes (Cobaugh *et al.*, 2015; Fancher *et al.*, 2017) in the pH of the underlying soil. With respect to the electrical conductivity of carrion-impacted soil, majority of the studies in the literature have reported an increase in soil electrical conductivity as decomposition progressed (Aitkenhead-Peterson *et al.*, 2012; Stokes *et al.*, 2013; Macdonald *et al.*, 2014; Fancher *et al.*, 2017; Yong *et al.*, 2019; Taylor *et al.*, 2020; Heo *et al.*, 2021).

The variability in the soil physico-chemical responses (e.g., pH) to cadaver decomposition may be attributed to the carrion type used in the study as well as the variation in regional- or country-specific environmental factors such as geology, topography, climate, soil type, series and texture, and soil microbiology (Tibbet and Carter, 2009; Aitkenhead-Peterson *et al.*, 2012, 2015; Tumer *et al.*, 2013; DeBryun *et al.*, 2021; Taylor *et al.*, 2023). For instance, the activities, reproduction, and development of carrion-associated insects, endogenous bacteria and enzymes are accelerated under moderately high temperatures (Spennemann and Franke, 1995; Campobasso *et al.*, 2001; Aufderheide, 2011). This, in turn, brings about an increase in the rate of decomposition, which is largely facilitated by the intense feeding activities of the larvae (Rodriguez and Bass, 1983; Spennemann and Franke, 1995; Campobasso *et al.*, 2001; Aufderheide, 2011). This consequently brings about the release of more decomposition fluids into the underlying and surrounding soil, and as a result, exerts a significant impact on the soil chemistry.

The amount of rainfall could also influence the changes in the chemistry of carrion-associated soils by interfering with the rate/pattern of decomposition. Moderate to heavy rainfall have been documented to confine the feeding activities of maggots to the internal tissues of vertebrate remains, increase the concentration of volatile organic compounds, disintegrate the flesh of the vertebrate remains, wash away fly eggs and larvae from the carcass (Anderson and Vanlaerhoven, 1996; Mahat *et al.*, 2009; Amendt *et al.*, 2010; George *et al.*, 2013; McLeod, 2015; Brownlow *et al.*, 2023; Ciésła *et al.*, 2023; Sluis *et al.*, 2023). All of these ultimately impact on the soil chemistry by influencing the amount of decomposition fluids seeping into the underlying soil from the carcass. Notwithstanding, changes in soil chemistry have been suggested to be useful in the estimation of the PMImin, detection of cadaver deposition sites and location of clandestine graves (Vass *et al.*, 1992; Carter *et al.*, 2009; Tibbet and Carter, 2009; Pringle *et al.*, 2010; Aitkenhead-Peterson *et al.*, 2012, 2015). These variations in soil physico-chemical responses to carrion decomposition highlight the need for the establishment of local, regional, and country specific baseline data on the changes in the chemistry of soils associated with decomposing cadavers.

1.7. Larval mass and carcass skin chemistry in terrestrial environments

As feeding and larval development continues, these larvae begin to form clusters in selected parts of the carrion, commonly referred to as maggot masses. Depending on the geographical location, stage of carrion decomposition, and season, the larval masses are usually variable in size, location of establishment on the carrion, and species composition (Rivers *et al.*, 2011).

Most research focusing on the larvae of forensically important flies has been conducted in relation to the heat generated within the larval masses and the effect of temperature on larval development, behavior and PMI estimations (see e.g., Byrd and Butler, 1997; Nabity *et al.* 2006; Slone and Gruner, 2007; Niederegger *et al.*, 2010; Charabidze *et al.*, 2011; Hückesfeld *et al.*, 2011; Rivers *et al.*, 2011; Johnson *et al.*, 2013; Heaton *et al.*, 2014; Kotzé *et al.*, 2016; Ahmad and Omar, 2018). Furthermore, some studies have investigated the volatile odor profile of larval masses, impact of different tissue types on the development of fly larvae, and the potentials of larval aggregations in medico-legal investigations such as the detection of drugs and toxic compounds, and sexual assault (Gagliano-Candela and Aventaggiato, 2001; Campobasso *et al.*, 2004; Day and Wallman, 2006; Byrd and Castner, 2010; Blonar and Prada-Tiedemann, 2020; Chamoun *et al.*, 2020).

Recently, forensic scientists have begun to explore the microbial community and chemistry (e.g., pH, oxidation-reduction potential and sodium ion concentration) of fly larval masses in a bid to provide additional sources of information that can supplement or enhance the accuracy of existing methods used in PMI estimations (Johnson *et al.*, 2013; Chun *et al.*, 2015; Weatherbee *et al.*, 2017; Dibner *et al.*, 2019; Junkins *et al.*, 2019; Kodama *et al.*, 2019). Some authors have observed no substantial successional changes in the microbial community of larval masses during carrion decomposition (Dibner *et al.*, 2019). However, other authors have found substantial changes and considerable trends in the microbial composition of larval masses during the early and late stages of decomposition and have highlighted the potential of these changes in relation to PMI estimation (Chun *et al.*, 2015; Junkins *et al.*, 2019).

Recent studies have shown with respect to fly larval mass chemistry that fly larval masses are largely an environment characterized by little to no oxygen (Chun *et al.*, 2015; Junkins *et al.*, 2019; Dibner *et al.*, 2019). In addition, acid to alkaline shifts (Junkins *et al.*, 2019), and conversely, alkaline to acid shifts (Chun *et al.*, 2015) in the pH of larval masses associated with decomposing carcasses have been reported. The acid to alkaline shifts in pH and vice versa may be attributed to the changes in the salivary and gut secretions produced during the feeding process of blow fly larvae as evidenced by studies on maggot therapy and their role in wound healing (Brocklesby, 2002). In fact, some studies have shown that in the presence of maggots, an acid to alkaline shift in wound pH was observed and this has been found to exert some antimicrobial activities on wound inhabiting bacteria (see Brocklesby, 2002). While these changes in larval mass microbiology and chemistry require further detailed investigations, they have been suggested to assist in the estimation of the PMI in the absence

of forensic entomological and taphonomic expertise (see Chun *et al.*, 2015). As with the changes in the chemistry of carrion-impacted soil, variations in the chemistry and microbial composition of fly larval masses may probably be linked to the type of fly species establishing the maggot mass(es), location of establishment of maggot masses, season of study, and environmental conditions (e.g., temperature, humidity, and rainfall) of the cadaver deposition site.

Apart from the fly larval masses in, on or around the vertebrate carrion, the carrion skin is also an interesting environment for microbial interactions and chemical changes (Pechal *et al.*, 2014; Chun *et al.*, 2015; Johnson *et al.*, 2016; Weatherbee *et al.*, 2017; Dibner *et al.*, 2019). Yet, there exists limited research on the post-mortem changes in chemistry of the vertebrate skin despite the tendency of the skin to persist until the terminal stages of decomposition and extended post-mortem periods (Dibner *et al.*, 2019). A study on the oxidation-reduction potential of the skin demonstrated that vertebrate carcass skin is a reducing environment with little to no oxygen (Dibner *et al.*, 2019). Furthermore, the authors reported high sodium concentration and an alkaline to acid shift in the pH of the carcass skin as decomposition progressed (Dibner *et al.*, 2019).

1.8. Forensic entomology and soil chemistry in PMImin estimation

The accurate estimation of the PMI is crucial in medico-legal investigations of death (Sharma *et al.*, 2015; Madea, 2016). By virtue of the uniqueness of the circumstances associated with each death, no single scientifically proven method exists that can accurately and precisely estimate the PMI of an individual (Madea, 2016). However, the use of insects has been found to be the most accurate and reliable method in the estimation of the PMImin of an individual or animal after 72 hours of death where most biological cues used by medical practitioners would have disappeared (Anderson and Vanlaerhoven, 1996; Buchan and Anderson, 2001; Jarmusz *et al.*, 2020).

The PMImin can sometimes be used interchangeably with the time since insect colonization (TOC) or period of insect activity (PIA) (Byrd and Castner, 2010). These terminologies are very similar, and some authors opined that it is up to the forensic entomologists to differentiate between these closely related descriptions of the time since death using readily available weather and environmental data (Byrd and Castner, 2010). In some forensic entomological cases, the PMImin or time since colonization is often within the range the individual was last seen to be alive or murdered when using early arriving insects (e.g.,

blow flies, flesh flies or house flies) as entomological evidence in the PMI estimations (Introna *et al.*, 1998; Pai *et al.*, 2007; Niederegger and Mall, 2021). However, this is dependent on presence/absence of physical barriers (e.g., suitcases, clothing, trashcans etc.) that can inhibit or delay the colonization and subsequent oviposition of blow flies or flesh flies on the vertebrate remains (Byrd and Castner, 2010). Insects can be utilized in estimating the time since death in two complementary ways by: (a) summing up the time it takes to attain the insect developmental stage recovered from the corpse in relation to the stage of decomposition and ambient climatic factors and (b) observing the insect community and their pattern of succession on the corpse (Byrd and Castner, 2010; Amendt *et al.*, 2011; Vanin and Huchet, 2017).

1.8.1. Insect age estimation

This method is used in the estimation of shorter PMIs usually within several days to weeks (Vanin and Huchet, 2017). It focuses on the developmental stages of immature (e.g., larvae and pupae) flies belonging to the families Calliphoridae, Sarcophagidae and Muscidae found on a deceased body. By deducing the age of the oldest developmental stage of the fly species recovered from or around the body in relation to the temperature exposure, an estimate of the PMImin or time since insect colonization can be obtained (Vanin and Huchet, 2017). Prior to the utilization of the developmental stages of insects associated with vertebrate carrion in PMImin estimations, adequate knowledge about the species, its lifecycle and the environmental factors that influence it is crucial (Vanin and Huchet, 2017; Finaughty, 2019). Most insect species of forensic importance employed in PMImin estimations are characterized by a holometabolous (i.e., complete) life cycle. This involves the transition across four developmental stages, commencing from the egg stage to the larval, pupal, and then adult stage (Byrd and Castner, 2010).

The duration of each developmental stage is dependent on the insect species, food availability, and the surrounding environmental factors (Finaughty, 2019). Due to the poikilothermic nature of insects, their development is largely tied to the temperature of the immediate environment (Byrd and Castner, 2010; Harvey *et al.*, 2016; Finaughty, 2019). Therefore, as the temperature of the environment increases, their metabolic rate increases which in turn speeds up development (Agrawal *et al.*, 2021). Owing to this, the duration of development of an insect decreases in a near-linear manner with increasing environmental temperature (Agrawal *et al.*, 2021). By examining the relationship between insect development and temperature, models such as the isomorphen, isomegalen and thermal summation models

can be used to predict the age of insect specimens of forensic importance (Amendt *et al.*, 2011; Harvey *et al.*, 2016).

Isomorphen models are simple scatter plots of the duration of specific insect developmental events on the x-axis plotted against temperature on the y-axis (Amendt *et al.*, 2011; Harvey *et al.*, 2016). By deducing the stage of development of the oldest immature insects found in, on or around the body, and the average ambient temperature of the scene of death while the body was still there, an estimation of the PMImin from the isomorphen model can be carried out (Amendt *et al.*, 2011). The simplicity of this model makes it advantageous, however, the drawback is that the accuracy is affected by fluctuations in ambient temperature (Amendt *et al.*, 2011). An additional drawback to the utilization of the isomorphen model is that it shows no gradation between developmental events as it only models the timing of developmental events (Amendt *et al.*, 2011). Equally important, there is a need for the specimen to be kept alive post-collection and reared to the next development event at a known and constant temperature, and this is usually uncommon (Amendt *et al.*, 2011).

To account and accommodate for the possibility of utilizing dead entomological specimens recovered from decomposing vertebrate remains alongside fluctuating ambient temperatures, a more sophisticated approach, is the isomegalen diagram, was developed. Here, the size of the larva (deduced from its length, weight, or width) instead of the life stage is plotted against temperature and time (Amendt *et al.*, 2011; Harvey *et al.*, 2016). In comparison to specific developmental events, the larval size produces a higher resolution of the age of the larval specimen, and thus a more accurate representation of the PMImin can be obtained (Amendt *et al.*, 2011; Harvey *et al.*, 2016). In spite of this, the isomegalen diagram is not without some drawbacks. These include the inconsistencies in larval size and age, likelihood of larval shrinkage prior to transition to the next developmental stage and/or during preservation, and larval stuntedness due to the unavailability of food and competition with other larvae (Amendt *et al.*, 2011; Harvey *et al.*, 2016).

The third type of model, thermal summation, is more complex (Amendt *et al.*, 2011). In the forensic context, this model is the most used method for the modelling of insect developmental rates and PMImin as it combines the size of an insect larva, stage of development and the fluctuating ambient temperatures into one linear regression model (Amendt *et al.*, 2011). In this model, the relationship between the developmental rate of an insect and the temperature of its surrounding environment is assumed to be linear especially

within the minimum and maximum developmental thresholds, and anything above or below these thresholds would invariably lead to the cessation of development and/or death of the insect (Amendt *et al.*, 2011). Insect development is measured in terms of thermal time (i.e., temperature multiplied by time in hours/days) with the transition from one developmental stage to another occurring at a given physiological and energy cost expressed in Accumulated Degree Hours (ADH) (Amendt *et al.*, 2011). Thus, by calculating the duration it takes to reach the ADH required for a specific insect species to transition into the specific stage of development recovered from the vertebrate remains, an estimate of the time since insect colonization of an individual can be obtained based on published development data (Amendt *et al.*, 2011).

1.8.2. Insect succession

This method is useful in the estimation of longer PMIs usually after several months and years (Amendt *et al.*, 2011; Vanin and Huchet, 2017). It capitalizes on the established fact that insects colonize decomposing vertebrate remains in a predictable sequence referred to as succession (Anderson and Vanlaerhoven, 1996; Cruise *et al.*, 2018), and that this sequence varies with respect to the season of death and geographical location of the corpse (Goff, 1993; Vanin and Huchet, 2017). Prior to the application of this method in PMImin estimations, detailed knowledge and understanding of the insect species attracted to decomposing vertebrate remains across the various decomposition stages in each season and geographical location is crucial. In this method, the PMImin can be established by associating the entomological specimens recovered from decomposing vertebrate remains at a given stage of decomposition, season, and geographical location to previously established local or region-specific baseline database of insect species attracted to decomposing vertebrate remains at various stages of decomposition. From this association, a broad timeline of the PMImin can be generated (Goff, 1993).

In the forensic entomology literature, studies focusing on the establishment of baseline data on the diversity, abundance and successional patterns of insect species attracted to decomposing vertebrate remains across several geographical locations have been conducted (Byrd and Castner, 2010; Matuszewski *et al.*, 2020). Owing to the substantial logistics and ethical difficulties associated with the use of human cadavers in establishing baseline insect successional data internationally, several studies have created valuable data using animal models (see Matuszewski *et al.*, 2020). Among all these animal models, the domestic pig has been regarded as the most suitable animal model for decomposition in entomology research (Whitaker, 2014; Mole and Heyns, 2019; Matuszewski *et al.*, 2020). Several justifications for the use of porcine models exist. These include their availability and relatively cheap cost;

acceptance of the use of pigs in research by the public and scientific community; ability to control for variability associated with size, sex, and diet; and similarities in the integumentary, cardiovascular, and gastrointestinal systems of pigs compared to humans (Catts and Goff, 1992; Campobasso *et al.*, 2001; Whitaker, 2014; Matuszewski *et al.*, 2020).

Logistical and ethical constraints may limit the ability to perform real-time decomposition studies. In such circumstances, forensic entomological and decompositional data may be obtained retrospectively through case file analysis (Cameron, 2016; Finaughty, 2019; Baliso *et al.*, 2019, 2023). By doing this, information such as date and place of corpse discovery, sex and age of decedent, decomposition stage of the cadaver, particularities of the place of corpse discovery, and associated insect species and life stages can be obtained (Lutz *et al.*, 2021a; Baliso *et al.*, 2019, 2023). Several published retrospective studies exist summarizing the diversity and abundance of forensically important insect species associated with human cadavers at different stages of decomposition, with varied manner of death, and in different seasons, ecological habitats, and scene types (see Sukontason *et al.*, 2007; Kavitha *et al.*, 2013; Sanford, 2017; Abdullah *et al.*, 2017; Lutz *et al.*, 2021a; Palavesam *et al.*, 2022). The advantage of conducting retrospective forensic entomological and decompositional studies is that they are inexpensive in comparison to experimental studies and can provide valuable insights not only on what aspects require considerable attention but also, areas that require further investigation during experimental studies in a specific region (Finaughty, 2019; Baliso *et al.*, 2019, 2023). Despite the benefits associated with conducting retrospective studies, Finaughty (2019) succinctly highlighted the difficulties and challenges associated with such studies. These include access to the forensic case files due to issues around sensitivity and confidentiality, incomplete or poorly completed case records, and low sample sizes.

The use of animal models as proxies for human cadavers has generated substantial data that have been instrumental in understanding the patterns and rate of decomposition and entomological activities around decomposing vertebrate remains (Dawson *et al.*, 2020, 2021a). However, some debate exists on the applicability and extrapolation of the data generated using animal models to human forensic cases (Schoenly *et al.*, 2007; Whitaker, 2014; Dawson *et al.*, 2020; DeBryun *et al.*, 2021). In the forensic entomological context, some studies have shown that decomposing human and pig remains host similar insect assemblages (Schoenly *et al.*, 2007; Whitaker, 2014; Matuszewski *et al.*, 2020). However, other studies have revealed significant differences in the insect communities between decomposing human and pig remains (Dawson *et al.*, 2020).

Empirical evidence indicating significant variation in soil biochemical responses to the decomposition of human cadavers and pig carcasses exist (Barton *et al.*, 2020; DeBryun *et al.*, 2021). For example, DeBryun *et al.* (2021) revealed that the pH of soils underneath decomposing human cadavers decreased while those underneath decomposing pig carcasses increased. The authors observed significantly higher ammonium and protease activities in the soils underneath the pig carcasses when compared to those of the human cadavers. Other studies have also shown significant dissimilarities in the rate and pattern of decomposition between human and pig remains, and the volatile organic compounds dissipated by both mammalian species (Rosier *et al.*, 2015; Connor *et al.*, 2018; Dautartas *et al.*, 2018; Dawson *et al.*, 2020; DeBryun *et al.*, 2021). These variation in insect communities, soil biochemical responses and decomposition processes between human cadavers and pig carcasses may be attributed to the differences in the moisture content, nitrogen content, saturated fatty acids, volatile organic compounds, and microbial composition between the two mammalian species (Carter *et al.*, 2007; Dawson *et al.*, 2020; DeBruyn *et al.*, 2021).

1.8.3. Changes in soil chemistry

Carrion impacted soil chemistry analysis can generate useful data that can complement entomological-based PMImin estimations during the first 72 hours following death (Aitkenhead-Peterson *et al.*, 2015). Soil chemical analysis can also be useful in PMImin estimations of decomposition cases especially during the late stages of decomposition where immature entomological evidence is unavailable due to post-mortem larval migration or where insect presence or access is hindered (e.g., when a body is buried) (Aitkenhead-Peterson *et al.*, 2015). Studies investigating the changes in the chemistry of the soils associated with decomposing human and animal remains are not uncommon (Singh *et al.*, 2023). However, studies focusing on the application of soil chemistry in PMImin estimations are limited (Aitkenhead-Peterson *et al.*, 2015).

Vass *et al.* (1992) successfully generated baseline data on the changes in the concentrations of volatile fatty acids, anions and cations in the soil associated with seven fresh, un-embalmed decomposing human cadavers. The authors were able to apply the changes in the concentration of these soil chemical properties to estimate the PMI of deceased individuals found decomposing in terrestrial environments. In contrast to the study by Vass *et al.* (1992), Carter *et al.* (2009) was able to use the concentration of ninhydrin-reactive nitrogen in the soils within the vicinity of a partially decomposed, scavenged, and scattered remains of an individual whose death was linked to suicide in identifying the exact location of death and site of

decomposition. According to the authors, the levels of ninhydrin-reactive nitrogen within the site of decomposition was 7 to 13 times higher than in other areas of the death scene.

1.9. Research rationale

In South Africa, law enforcement agencies and forensic mortuaries are often confronted with a staggering number of suspected unnatural deaths and unidentified individuals (Evert, 2012; Reid *et al.*, 2019, 2020, 2023; Keyes *et al.*, 2022). Furthermore, homicide - and notably murder (intentional/aggravated homicide) - is an acute socio-criminal issue, exemplified by South Africa having one of the highest murder rates internationally (SAPS, 2023; Matzopoulos *et al.*, 2015; Pillay-van Wyk *et al.*, 2016; Baliso *et al.*, 2019). Homicide is currently identified as one of the leading causes of fatal injuries and unexplained deaths within the country (Lerer *et al.*, 1997; Norman *et al.*, 2007; Groenewald *et al.*, 2008; Mathews *et al.*, 2009; Cocks and Saayman, 2013). Owing to this, an accurate estimate of the PMI is critical for forensic death investigation as it narrows the pool of missing persons to identify the decedent and can help refute or corroborate witness or suspect testimony (Dirkmaat *et al.*, 2008; Megyesi *et al.*, 2005; Sutherland *et al.*, 2013). During medico-legal forensic investigations, all possible sources of evidence in, on and around the human remains and death scene needs to be included when attempting to solve these cases and possibly explain the circumstances that led to the death of the individual. Some of this evidence includes cadaver-associated insects and soil samples (Niederegger and Mall, 2021; Singh *et al.*, 2023).

While several research studies have investigated the necrophagic arthropod diversity, abundance, and successional patterns on decomposing vertebrate remains in South Africa, there has been limited work in the Western Cape Province (see references listed in section 1.4, Prins, 1982; Finaughty, 2019). To date, Finaughty (2019) is the sole study that has categorically investigated the diversity, abundance, and successional patterns of forensically important insect species on decomposing vertebrate remains in the Western Cape. The Western Cape Province is biogeographically unique (Rebelo *et al.*, 2006). The Province is characterized by a considerably high amount of invasive plant species when compared to other Provinces (Richardson *et al.*, 2020). Furthermore, within the Province is the biologically diverse Cape Floral Kingdom (CFK), which is one of the world's six plant kingdoms (Pooley, 2010; van Wilgen *et al.*, 2012). The summers within the CFK are long, hot, windy, and dry, while the winters are wet (Pooley, 2010). Approximately 9,000 plant species exist in the CFK, of which 69 per cent are endemic (Pooley, 2010). The CFK is largely dominated by the Mediterranean-type shrubland called Fynbos, which is adapted and prone to fire (Pooley, 2010; van Wilgen *et*

al., 2012). Equally important, the Western Cape is unique due to the consistent and continued fluctuations in topography, climate (e.g., rainfall), and ocean currents (Goldblatt and Manning, 2002; Pooley, 2010; CSIR, 2014; Baliso *et al.*, 2023).

Thus, baseline insect diversity and successional data from experimental field studies in other Provinces cannot be directly applied to actual forensic cases dealing with insects in the Western Cape Province, consequently necessitating the need for the establishment of a region-specific baseline data of insect species associated with decomposing carcasses. Although Finaughty (2019) has established the first baseline data on carrion-associated insect species in the summer and winter seasons of the Western Cape Province, his study was limited to the open and thicketed Cape Flats Dune Strandveld regions. Furthermore, the entomological findings of Finaughty (2019) may not completely depict the entire insect fauna attracted to decomposing vertebrate remains within the Province owing to the substantial variation in the climate type, topography, and surrounding ocean currents in several parts of the region (Goldblatt and Manning, 2002; Baliso *et al.*, 2023). This speculation can be validated by some studies in the literature where considerable differences in the insect assemblages associated with decomposing vertebrate remains in different geographical areas or localities within the same region have been reported (Cruise *et al.*, 2018; Díaz-Aranda *et al.*, 2018; Stone *et al.*, 2023). This further warrants the need for not only region-specific, but also, local, and environment-specific baseline forensic entomological studies.

Table Mountain National Park, which is one of the World Heritage Sites on account of its exceptional plant and invertebrate biodiversity (Cowling *et al.*, 1996; van Wilgen *et al.*, 2012). Table Mountain National Park attracts local and international tourists and visitors, and over 3.1 million visitors were reported to have visited the park in 2019 (Cape Town Regional Trends, 2019). As the National Park attracts many visitors, cases of accidents and casualties within and around the Park are not uncommon (Maistry, 2015; Baliso *et al.*, 2019). Maistry (2015) conducted a retrospective analysis of deaths in the Table Mountain National Park between 2000 – 2011, and reported a total number of 98 deaths, with most of the deaths occurring during the summer months. In addition, unnatural deaths as a result of murder (via gunshots, assaults, and strangulation), suicides (via hanging, cliff jumping, gassing), heatstroke, and drug overdose within the National Park were reported by the author. For these reasons, the Table Mountain National Park is an environment of forensic interest, and there is scope for entomological evidence from cases of death in the confines of Park and its immediate environments. To the best of the author's knowledge, no published studies exist on the diversity,

abundance, and successional patterns of insects on decomposing carcasses within the Table Mountain region of the Western Cape Province.

In the forensic entomology literature, a plethora of studies in various parts of the world have investigated the diversity, abundance, and successional patterns of insects on a single or multiple decomposing carcasses in a single season (comprising of several months, usually two to three), between seasons, or across multiple seasons (see Braack, 1981, 1986, 1987; Kelly, 2006; Kolver, 2009; Gilbert, 2014; Botham, 2016; Martín-Vega *et al.*, 2017, 2019; Díaz-Aranda *et al.*, 2018; Shayya *et al.*, 2018b; Finaughty, 2019; Sebastião and Prado e Castro, 2019; Jarmusz *et al.*, 2020; Tembe and Mukaratirwa, 2021; Tembe *et al.*, 2021a, b, to mention a few). Yet studies investigating whether there are differences in the first insect colonizers, diversity, abundance, and successional patterns of insects on freshly deployed and complete decomposing vertebrate remains in each of the month constituting a single season are scarce.

In the United Kingdom, McGonigal *et al.* (2022) conducted a year-long study on the insect species attracted to baited traps and decomposing rabbit carcasses. However, their study only investigated the monthly variation (across all the seasons) in the diversity, abundance, and successional patterns of blow flies (Calliphoridae). Parry *et al.* (2016) in South Africa utilized baited modified fly traps to document the diversity and abundance of forensically relevant flies in different seasons and habitats. However, their study was limited to selected months in each of the four seasons in South Africa. Furthermore, results and conclusions from previous and recent studies such as that of Hepburn (1943) and LeBlanc *et al.* (2021) revealed that the use of baited fly traps (with small amount of animal tissues) in documenting the diversity and abundance of necrophagous flies in an environment and in forensic investigations should be done with caution as they do not give an accurate depiction of the fly species occurrence and general insect assemblage attracted to vertebrate carrion.

Despite several decomposition studies focusing on forensic entomology and taphonomy (Finaughty, 2019; Forbes *et al.*, 2019; Spies, 2022), and the substantial number of human cadavers found in different terrestrial environments (e.g., vegetated areas, roadsides, and farmlands) in different seasons within the Western Cape Province (Baliso *et al.*, 2019, 2023), no published studies exist on the impact of cadaver decomposition on the chemistry of the underlying soil across different seasons in the Western Cape Province and South Africa at large. Also, there is lack of empirical data on the decomposition rate/pattern and insect assemblages of the remains of infants or neonates despite their occurrence in medico-legal

investigations within the Province (Baliso *et al.*, 2019). Equally significant, no studies within the African continent have attempted to examine the changes in the chemistry of a vertebrate carcass skin and its associated larval mass(es) at different stages of decomposition.

Notable trends in soil-physicochemical responses to carrion decomposition, albeit inconsistent, have been established in other countries/continents (Singh *et al.*, 2023). However, as with insect diversity and succession patterns on decomposing vertebrate remains, the use of soil, carcass skin and larval mass chemistry data derived from different countries may lead to inaccuracies when employed in the estimation of the PMImin in South Africa. Also, due to the variation in weather conditions (e.g., temperature, and rainfall) between seasons and the subsequent impact on the process of decomposition and necrophagous insect activities even in a given locality (Byrd and Castner, 2010), it is acceptable to speculate that, as seasons change, there will be differences not only in the onset of changes in the chemistry of the soil beneath and around decomposing vertebrate remains, but also, changes in the chemistry of the carcass skin and larval mass pH.

1.10. Research aims and objectives

This study started off as a Master's (by Dissertation) research with the overall aim of establishing baseline entomological and soil chemistry data associated with decomposing neonate pig carcasses on a monthly and seasonal temporal scale in the Table Mountain region of the Western Cape Province in order to estimate the PMImin and the detection of previous cadaver deposition sites and clandestine graves. At the onset of the Master's field research, the primary objectives were to:

1. Determine the rate and pattern of decomposition in each month of the year for this region.
2. Determine the abundance, diversity, and successional pattern of forensically important insect species attracted to neonate pig carcasses across all the stages of decomposition in each month (January – December) and cumulatively, seasons (summer, autumn, winter, and spring) of the year for this region.
3. Determine the impact of neonate pig carcass decomposition on soil pH and electrical conductivity across all the stages of decomposition in each of the four seasons (summer, autumn, winter, and spring) for this region of South Africa.

4. Determine the changes in the pH of the neonate pig carcass skin and larval mass(es) (where present) across all the stages of decomposition in each of the four seasons (summer, autumn, winter, and spring) for this region of South Africa.

The completion of the monthly neonate pig decomposition trials across the spring and summer seasons resulted in the generation of some novel observations within the South African forensic taphonomy and entomology literature which facilitated the conversion/upgrade of the MSc. degree to a PhD degree. This allowed for further investigations and completion of the monthly neonate pig decomposition trials across the autumn and winter seasons. Equally important, in line with the quest for forensic relevance, specifically in relation to the generation of forensically relevant data that can be utilized in local forensic investigations (see Miles *et al.*, 2020), this study drew inspiration from the observations recorded in local forensic cases and published retrospective studies (e.g., Baliso *et al.*, 2019, 2023) within the Province, to develop secondary objectives for the thesis. The secondary objectives were developed in order to create baseline data that reflects what is generally obtainable in local forensic cases within the region and these were specifically to:

1. Determine the abundance, diversity, and successional pattern of forensically important insect species attracted to singly deployed adult pig carcasses across all the stages of decomposition in the winter and summer seasons of the year for this region and to verify if these variables differ in comparison to the data generated from neonate pig carcasses in similar seasonal trials.
2. Determine the impact of singly deployed adult pig carcass decomposition on soil pH and electrical conductivity across all the stages of decomposition in the winter and summer seasons of the year for this region and to verify if these variables differ in comparison to the data generated from neonate pig carcasses in similar seasonal trials.
3. Determine the changes in the pH of the adult pig carcass skin and larval mass(es) (where present) across all the stages of decomposition in the winter and summer seasons of the year for this region and to verify if these variables differ in comparison to the data generated from neonate pig carcasses.
4. Retrospectively investigate the insect species associated with decomposing human cadavers within the Western Cape Province from the Forensic Pathological Service Autopsy Office and Cape Forensic Taphonomy and Entomology Databases, and then compare with the insect data generated from field experimental studies using neonate and adult pig carcasses.

Chapter 2: Materials and Methodology

To investigate and generate baseline data on the assemblage and activities of carrion-associated insects alongside the impact of cadaver decomposition on soil chemistry, this study conducted field experimental trials utilizing neonate and adult pig carcasses as proxies for a human body. To examine how similar the insect assemblage documented on the pig carcasses were, to those collected from decomposing human remains within the Province, forensic entomological case reports were reviewed, and the insect species reported therein, were documented. This study was divided into four main components: namely (1) insect species and their successional patterns on decomposing neonate and adult pig carcasses, (2) insect species collected from decomposing human remains, (3) changes in the pH and electrical conductivity of soils underneath and around decomposing pig carcasses, and (4) changes in carcass skin and established larval mass(es) pH on the carcasses.

2.1. Study site

The trials in this study were conducted within the borders of the Table Mountain National Park (S33° 57.164', E018° 27.733'; S33° 57.827', E018° 27.464'; S33° 57.682', E018° 27.301'; S33° 57.6662', E018° 27.331'), adjacent to the University of Cape Town's, Upper Campus in the City of Cape Town, Western Cape Province, South Africa (Figures 2.1 & 2.2). The Table Mountain National Park is 265km² in area. It is a mountainous area characterized by a rocky topography (Forsyth and van Wilgen, 2008; van Wilgen *et al.*, 2012; Poulsen and Hoffman, 2015, Adetimehin *et al.*, 2022, 2023a). In 2004, the Table Mountain National Park was granted a World Heritage Site status on account of its exceptional plant and invertebrate biodiversity (Cowling *et al.*, 1996; van Wilgen *et al.*, 2012). In addition, it was inaugurated as one of the New 7 Wonders of Nature in 2011 (Maistry, 2015).

The predominant plant within the National Park is the Mediterranean shrubland called fynbos, a major constituent of the Cape Floristic Kingdom, which is reliant on, and prone to fire (van Wilgen *et al.*, 2012; Adetimehin *et al.*, 2022, 2023a). Other vegetation within the park includes Afromontane forests and renosterveld – a shrubland that is also prone to fire (van Wilgen *et al.*, 2011, 2012). In addition, the park harbors 2,285 plant species of which 90 are endemic species (van Wilgen *et al.*, 2012; Adetimehin *et al.*, 2022, 2023a). Compartments of invasive alien flora exist, dominated by *Acacia*, *Pinus*, and *Hakea* species (van Wilgen *et al.*, 2012; Adetimehin *et al.*, 2022, 2023a).

The elevation of the park ranges from sea level to 1086 meters with mean annual rainfall around 1,700mm in the north and 400mm in the south (Forsyth and van Wilgen, 2008; van Wilgen *et al.*, 2012). Within Table Mountain National Park, the summers are hot and characterized by dry winds with occasional dry-season fires, while the winters are cool and wet, characterized by frequent rainfall (Forsyth and van Wilgen, 2008; van Wilgen *et al.*, 2012). The dominant type of soil in the northern region of Table Mountain National Park where all the study trials were conducted is clay soil (van Wilgen *et al.*, 2012). Soils within the fynbos region are poor in nutrients, with neutral to acidic pH values ranging from 4 to 7 (Richards *et al.*, 1997; Mager and Hui, 2012). The mean $\pm$ standard deviation elevation of the neonate and adult pig carcass deployment sites respectively were 94.4 ± 5.2 and 136 ± 2.83 meters.

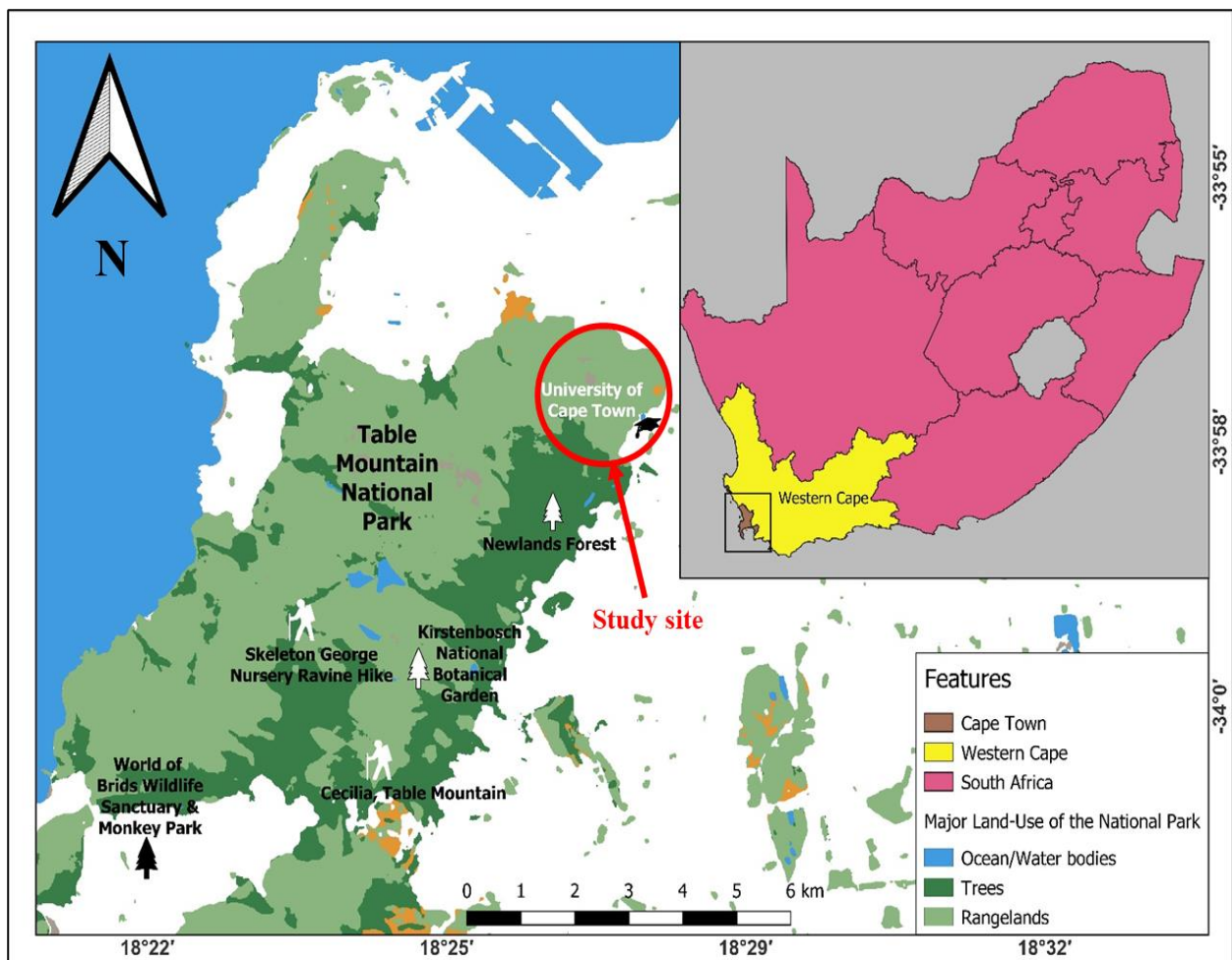


Figure 2.1: Map of South Africa showing the City of Cape Town within the Western Cape Province (top right), and the study site within the Table Mountain National Park highlighted by the red circle and arrow (center left).

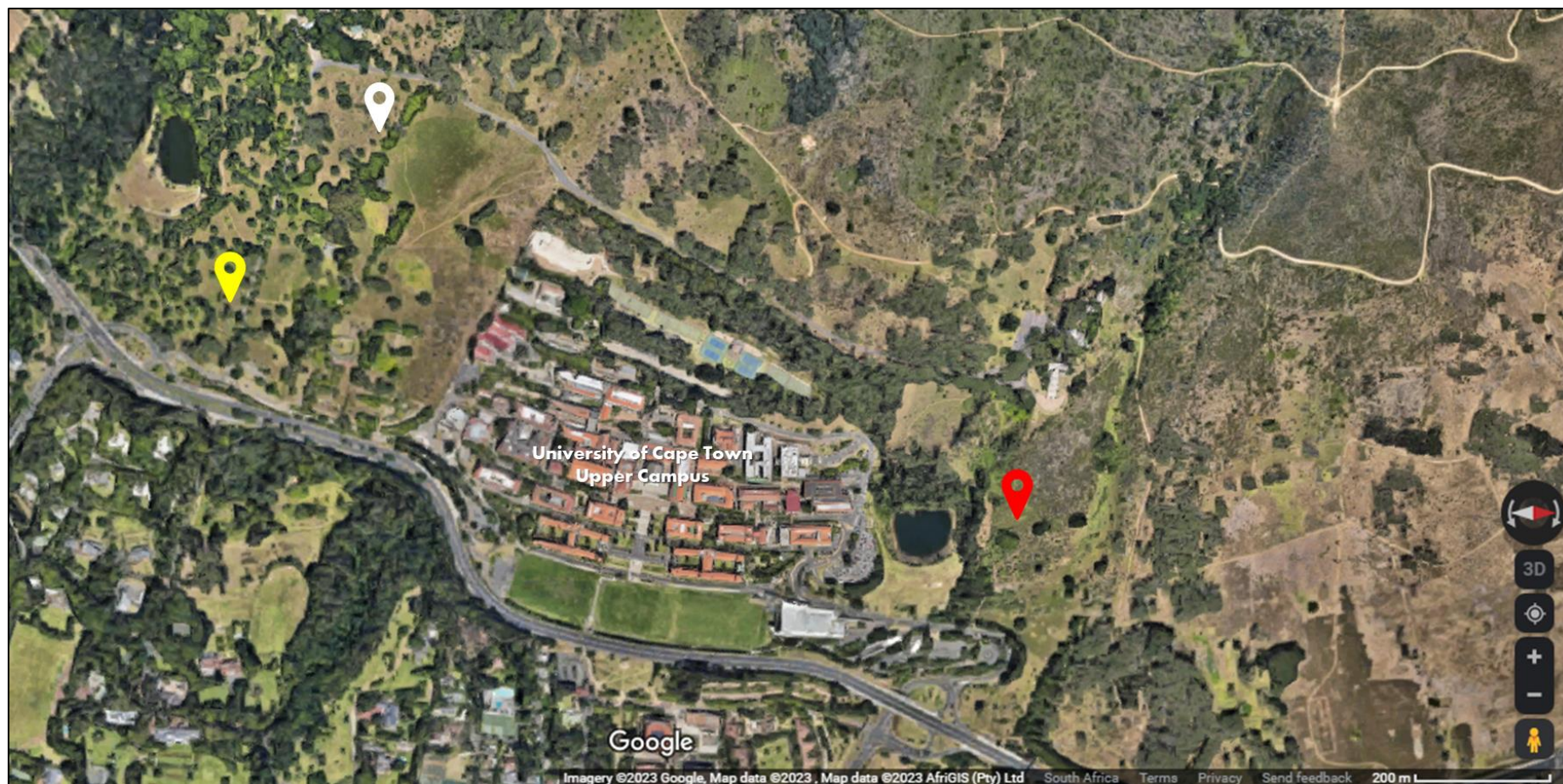


Figure 2.2: Aerial view of the University of Cape Town, Upper Campus, Rondebosch, Cape Town, and the surrounding area. Red mark point on the right-hand side of the University of Cape Town indicates the first neonate pig trials' study area within the Table Mountain National Park before the wildfires; Yellow mark point on the left-hand side of the University of Cape Town indicates the second neonate pig trials' study area within the Park after experimental setup relocation due to wildfires; White mark point on the upper left-hand side of the University of Cape Town indicates the adult pig trials' study area within the Table Mountain National Park (Adapted from Google Maps, 21st January 2022).

2.2. Animal Model

The animal model used for this study was the domestic pig (*Sus scrofa domesticus* L.). For the neonate pig trials, fresh-frozen stillborn pig carcasses were obtained from the Mariendahl Experimental farm, Elsenburg Agricultural Research facility, Stellenbosch University, after which they were transported to the Forensic Entomology Laboratory at the University of Cape Town Health Sciences Campus where they were further kept frozen at – 20°C prior to the commencement of the experimental trials. When required during the study, the stillborn pig carcasses were removed from the freezer, thawed overnight for a minimum period of 12 hours in a tightly sealed black plastic container after which they were rinsed with running tap water and then transported to the study site using the same container.

For the adult pig trials, two (2) 60kg domestic adult pigs were obtained from the Mariendahl Experimental farm, Elsenburg Agricultural Research facility, Stellenbosch University. Adult pigs were humanely terminated by a single shot to the base of the brain with a .22 caliber long rifle by the farm manager on the day of the commencement of the experimental trial, similar to the method applied by Finaughty (2019) and Spies (2022). Immediately following termination, the adult pig carcasses were rinsed with tap water and then sealed within a body bag for transport and deployment to the study site (Adetimehin *et al.*, 2022, 2023a). The adult pig carcasses were deployed on site within 2hrs of death.

2.3. Research trials and experimental configuration

2.3.1. Neonate pig trials

For the neonate pig trials, data were collected over four seasonal decomposition trials (spring, summer, autumn, and winter) commencing on the 13th of August 2020 and ending on the 24th of September 2021. Neonate pig seasonal trials commenced as close as practically possible to the winter/summer solstices and spring/autumn equinoxes of the southern hemisphere (Kelly, 2006, Adetimehin *et al.*, 2022, 2023a). The durations of each monthly neonate pig trial within a specific season are shown in Table 2.1. A total of 24 stillborn pig carcasses were utilized for the entire neonate study. During each calendar month per season, two (2) freshly thawed stillborn pig carcasses were placed individually into a partially open-bottomed plastic basket (length = 42cm, width = 25cm, and height = 6cm) positioned inside a rigid steel cage (length = 69cm, width = 53cm, height = 62cm) (Figure 2.3). The carcasses were in contact with the soil through openings (aperture size of 1.5cm in diameter) in the base of the baskets. The plastic baskets made it possible to collect insect specimens directly underneath the carcasses with less interference of the carcasses. At the time of experimental design, the inter-carcass distance was

of a minimum of 25m similar to Cruise *et al.* (2018). However, recent studies have suggested the utilization of an inter-carcass distance of 50m (Matuszewski *et al.*, 2020).

The steel cages were made of an outer rigid steel mesh (size = 2cm) fortified with double-layered chicken wire (mesh aperture size of 1.5cm in diameter) specifically to prevent the carcasses from being stolen while they were still fresh and to further prevent vertebrate scavengers that are capable of burrowing or digging (e.g., rats, birds, mongooses, and snakes) from having any contact with the carcasses. Within each of the steel cages, a medium sized tent-like double-layered brown net with a fine mesh was hanging above the carcass. The nets were held firmly to the edges of the plastic baskets using an elastic band. Two circular holes (4cm in diameter) were made at selected points of the net (just above the elastic band) to enable adult flying insects to gain access to the decomposing carcasses. The experimental steel cages were secured firmly to the soil using long stakes. Ground-crawling insects attracted to or moving away from the carcasses were trapped using pitfall traps buried at a minimum distance of 1m from the cages along four cardinal points (north, south, east, and west). The pitfall traps consisted of two 300ml transparent plastic containers placed inside each other and then filled with water and soap. The date of carcass placement was designated as Day 0 mostly between 10h00 – 11h00. Prior to the departure from the study site (i.e., Day 0), adult insects seen on the carcasses after their deployment were counted and recorded. However, if no insects were observed, collections of field/experimental data began at the same time, 24 hours post-carcass placement (Day 1). Insect collection and carcass examination were carried out daily where possible until the termination of the experimental trials. Data collection was not possible on some days due to unfavorable weather conditions (e.g., excessive rainfall), transport-related constraints, and fatigue/illness on the part of the researcher.

Table 2.1: Onset and duration of each monthly neonate pig trial in each season of the Western Cape Province.

Trial	Number of pigs	Mass of pigs in Kg (mean (SD))	Duration (Days)
Spring (Equinox: 21 st Sept)			
<i>Trial 1 (September 2020)</i>	2	1(0.07)	34
<i>Trial 2 (October 2020)</i>	2	1(0.35)	24
<i>Trial 3 (November 2020)</i>	2	1.1(0.07)	16
Summer (Solstice: 21 st Dec)			
<i>Trial 1 (December 2020)</i>	2	0.8(0.00)	10
<i>Trial 2 (January 2021)</i>	2	1.8(0.21)	13
<i>Trial 3 (February 2021)</i>	2	1.4(0.14)	13
Autumn (Equinox: 22 nd Mar)			
<i>Trial 1 (March 2021)</i>	2	1.1(0.14)	13
<i>*Trial 2 (May 2021)</i>	2	1.2(0.00)	29
<i>Trial 3 (May 2021)</i>	2	1.2(0.14)	35
Winter (Solstice: 22 nd June)			
<i>#Trial 1 (August 2020)</i>	2	1.4(0.07)	48
<i>Trial 2 (June 2021)</i>	2	1(0.07)	50
<i>Trial 3 (July 2021)</i>	2	1.9(0.07)	66

*Trial start delayed due to wildfires in the area; #First winter trial conducted in August 2020 but referred to as late winter trial (Adapted from Adetimehin *et al.*, 2022).



Figure 2.3: Close up view of the neonate pig carcass in the experimental cage (A), tent-like brown net hanging above the neonate pig carcass within the experimental cage (B) and complete experimental setup (C) within the vicinity of the Table Mountain National Park.

2.3.2. Adult pig trials

For the adult pig trials, data were collected over two seasonal decomposition trials (winter and summer) commencing on the 21st of June 2022 and ending on the 2nd of January 2023. The winter adult pig trial commenced on the day of the winter solstice (21st of June). Due to some constraints in logistics, the summer adult pig trial commenced on the 15th of December 2022, six days prior to the onset of the summer solstice (21st of December). The durations of the winter and summer adult pig trials are shown in Table 2.2. A total of two adult pig carcasses were utilized for the adult pig study. In each seasonal trial, one freshly killed adult pig (as described in the “Animal model” section) was placed inside a rigid steel cage. The adult pig carcasses were in direct contact with the soil. The sites of deployments for the winter and summer adult pig carcasses were a minimum of 25m apart to ensure there was no overlapping of previously existing crawling insect populations attracted to each carcass (Cruise *et al.*, 2018).

The steel cages used were of the same material as that of the neonate pig trials, however, it was enlarged to accommodate an adult pig carcass (length = 138cm, width = 62cm, height = 53cm) (Figure 2.4). The experimental setup for the adult pig carcasses was largely similar to that of the neonate pig carcasses. However, two circular holes (4cm in diameter) were made at selected points on each net hanging above the carcass to enable adult flying insects to gain access to the decomposing carcasses. Furthermore, there were no partially open-bottomed plastic baskets directly underneath each carcass. The date of carcass placement was designated as Day 0 and was between 10h00 – 12h00. Before leaving the carcasses’ deployment sites (i.e., Day 0), the number of all adult insects that were observed to colonize the carcasses was documented. In the absence of any insect, data collection began on Day 1. Insect collection and carcass examination where possible and as with the neonate pig trials, data collection was not possible on some days during these trials due to the circumstances mentioned above.

Table 2.2: Onset and duration of each adult pig trial in the winter and summer seasons of the Western Cape Province.

Trial	Number of pigs	Mass of pigs in Kg	Duration (Days)
Winter (Solstice: 21 st June)			
<i>Trial 1 (21st June 2022)</i>	1	59.6	149
Summer (Solstice: 21 st Dec)			
<i>Trial 1 (15th December 2022)</i>	1	59.5	18



Figure 2.4: Close up view of the adult pig carcass in the experimental cage (A), double tent-like brown net hanging above the pig carcass within the experimental cage (B) and complete experimental setup (C) within the vicinity of the Table Mountain National Park.

2.4. Insect data

All adult insects found within the nets were collected daily by collapsing the nets, detaching them from the cages and then transferring the entire nets and their contents into a killing jar containing paper towels dampened with few drops of ethyl acetate (Adetimehin *et al.*, 2022, 2023a). Ethyl acetate is a highly volatile and non-persistent solvent (Löser *et al.*, 2014; Jaing *et al.*, 2021). As a result, the nets were aired for a minimum period of 10 minutes before the nets were replaced over the carcasses. This was to reduce the impact of the ethyl acetate that might be left on the nets specifically impacting on the attractiveness of the carcasses to carrion insects. In addition to the insects trapped in the nets, the number of visually recognizable individual insect taxa (i.e., species, family, and/or order) seen on or around the carcasses were also recorded.

Small batches of fly eggs and samples of about 5 – 10 individuals of fly larvae present on the carcasses during data collection were collected manually with the aid of soft tweezers and micro spoons, and placed in labelled screw top containers. Insects were transported to the Forensic Entomology Laboratory at the University of Cape Town's Health Sciences Campus for rearing and further analysis.

Upon arrival at the laboratory, the immature insects were placed on freshly cut pig livers in labelled containers filled with vermiculite after which the containers were kept in a Memmert climate chamber (Lasec, Serial Number: W6200065) (temperature: 25°C; relative humidity: 65%; photoperiod: 14:10) for rearing. The collection and rearing of the immature insect specimens to adults was to confirm the identity of the fly species that deposited its eggs or larvae on the carcasses. Following the emergence of the insects, the adult flies were terminated as described above, and identified to the family, genus or species level using the published identification keys and descriptions (Byrd and Castner, 2010; Lutz *et al.*, 2018).

Ground-crawling adult and immature insects found in, on and around the carcasses and in the pitfall traps were manually collected using soft tweezers, plastic spoons and sometimes a hand shovel. All collected adult insect specimens were sorted, counted, and identified either to the family, genus or species level using published morphological identification keys and descriptions (Wall *et al.*, 2008; Byrd and Castner, 2010; Lutz *et al.*, 2018; Picker *et al.*, 2019) under a Zeiss Digital Stereo Microscope (Discovery V20) and Zeiss Stemi Microscope (Stemi 508). Under certain circumstances when the family, genus or species of an insect specimen could not be readily determined by the researcher, Z-stacked images and, occasionally, samples

of the original specimens were sent to selected entomological experts within South Africa for accurate identification.

2.5. Decomposition data

Daily observation of the stillborn and adult pig carcasses was done visually, and associated video recordings and photographs were taken. The decomposition stage of the carcasses was determined using the revised total body scoring (TBS) system for pigs developed by Keough *et al.* (2017). The decomposition process was divided into four stages including, fresh (TBS = 3), early decomposition (TBS 4 – 16), advanced decomposition (TBS 17 – 24) and skeletonization (≥ 25) stages.

Each monthly and seasonal trial was terminated at the same end point following the onset of skeletonization. The end point was not defined based on elapsed time but rather defined using the criteria listed in Adlam and Simmons (2007: 1009), including (a) "obvious loss of internal abdominal structure, spine only remaining underneath dried skin, (b) substantial un-weathered bone exposed ($>50\%$) and no wet decomposition when observed underneath, (c) significant areas ($>30\%$) of bleached or weathered bone exposed."

2.6. Soil pH and electrical conductivity

Reference soil samples were collected from the study sites prior to the placement of the carcasses for Munsell Soil Color Book (2009). In all the decomposition trials, experimental soil sample collection for chemical analysis began on day 2 after the deployment of the carcasses. More specifically, in the neonate pig summer and autumn seasonal trials, soil samples were collected daily from Day 2 due to an anticipated rapid decomposition process. In the neonate pig spring seasonal trials, collection was conducted every second day except for the late spring trial (i.e., November trial) where data collection was done every day due to noticeable increase in ambient temperatures. In the neonate pig winter seasonal trials, soil samples were collected daily after the first collection. In some instances, data collection was done every second day depending on the rate of decomposition, logistics and ambient climatic factors.

To reduce the effect of frequent sample collection and its possible impact on the aeration of the soil, and potentially on, soil pH and electrical conductivity, soil sample collection in the adult pig summer trial was done every second day after the first collection, while in the winter trial, collection was done every third day after the first collection.

Soil samples were collected from two locations associated with the decomposing carcasses and stored in plastic screw top containers until analysis. These samples included a test sample from directly underneath the trunk of the carcass and a control sample collected 2 meters uphill from the carcass (Benninger *et al.*, 2008). Approximately 1g of soil was collected at each location and included soil up to a depth of 5cm (Benninger *et al.*, 2008).

For analysis, a 1:5 by volume soil:water mixture was created by dissolving 1g of the collected soil sample in 5ml of distilled water in a 50ml centrifuge tube (Benninger *et al.*, 2008). The soil-water mixture was then vortexed for 60 seconds to allow thorough admixture. Thereafter, the soil-water mixture was allowed to settle for 5 minutes after which pH and electrical conductivity was measured using a calibrated ThermoScientific Multimeter (Orion Star A329) with pH and electrical conductivity probes. To prevent cross contamination, the pH and electrical conductivity probes were rinsed twice with distilled water and then blotted with clean paper towels between each measurement.

2.7. Skin and larval mass(es) pH

Daily carcass skin pH in each of the trials was measured *in-situ* using a calibrated ThermoScientific Multimeter (Orion Star A329) with a pH probe. This was accomplished by inserting the probe in the region between the forelimbs and/or hindlimbs of the carcasses (Figure 2.5A). To prevent cross contamination, the pH probe was rinsed twice with distilled water and then blotted with clean paper towels between each measurement. The frequency of collection was the same as described in the “Neonate pig trials” and “Adult pig trials” sections. Following the establishment of the larval mass(es) in or on the carcasses, the pH of the mass(es) was measured *in-situ* using the same Multimeter. This was done by carefully inserting the probe into the center of the larval mass(es) (Figure 2.5B). Where there were multiple larval masses on the carcass, the pH probe was rinsed twice with distilled water and then blotted with clean paper towels between each measurement to prevent cross contamination.



Figure 2.5: Carcass skin (A) and larval mass (B) pH measurement with the ThermoScientific Multimeter with connected pH probe within the vicinity of the Table Mountain National Park.

2.8. Weather data

Internal and external temperatures of the stillborn and adult pig carcasses were recorded using iButton® sensors (Measurement Systems, Berkshire, United Kingdom). To measure the internal temperature, an iButton® sensor was inserted deep down the throat through the mouth while the external temperature was recorded by placing an iButton® sensor next to the carcass. The iButton® sensors were programmed to record temperatures every 60 minutes. Daily ambient temperature and relative humidity were recorded with the aid of a weather station positioned at the Department of Biological Sciences, Faculty of Sciences, University of Cape Town. The distance between the weather station and the study site is approximately 2km. Daily rainfall data was obtained from the nearest weather station situated in Newlands, Rondebosch, which is about 5km from the study site.

For each trial, the Accumulated Degree Days (ADD) were calculated for the temperatures recorded by the internal and external iButton® sensors. This was achieved by first averaging the temperatures recorded by the iButton® sensors for each day, then summing the daily average temperatures for each pig carcass (Dawson *et al.*, 2020). In the adult pig

summer trial, a Tiny Tag (TGP – 4500) weather data logger was used to take recordings of the carcass external temperature. Due to repeated battery and logger failures, some gaps were noted in the ambient temperature and relative humidity data recorded by the weather station, in some trials. Consequently, Generalized Additive Models (GAMs) as recommended by Lutz and Amendt (2020) were used to reconstruct the temperature and relative humidity for the missing days using similar data obtained from the weather history database of the Cape Town International Airport (33.97°S, 18.6°E), about 16km from the study site. Temperature and relative humidity data from the Cape Town International Airport alongside the hour of the day (0 to 23) were included as smooth terms in the GAM models. It should be noted that, in some trials, no carcass internal temperature data was recorded by the iButton® and this was likely due to the corrosion, or damage of the iButton® by the carcass decomposition fluids or by rainfall. This is not an uncommon occurrence and has been reported in other similar studies (Kelly, 2006; Cameron, 2016; Finaughty, 2019).

2.9. Retrospective insect data analysis from human forensic cases

The University of Cape Town offers forensic entomological consultation to Forensic Pathology Services through the Cape Forensic Taphonomy and Entomology Laboratory (CapeFORTE). A request for consultation and entomological analysis is made at the discretion of the consulting forensic pathologist assigned to a specific case.

A retrospective review of entomological reports produced by CapeFORTE between 1 January 2015 and 31 December 2022 was conducted. The purpose of this review was to document the adult and immature stages of forensically important insect species collected from decomposing human remains during autopsy and crime scene investigation within the Western Cape Province of South Africa. Where available, the following information from each entomological report was extracted: (1) date of discovery of the human remains, (2) place of discovery of the human remains, (3) sex of decedent, (4) stage of decomposition of the human remains, (5) insect species and life stage collected and identified, (6) place of entomological specimen sampling, and (7) collector of the entomological specimens. As documented in the reports analyzed, the collected insects were identified using published morphological descriptions and identification keys (Seago, 1953; Stojanovich *et al.*, 1953; Zumpt, 1965; Prins, 1982; Grassberger *et al.*, 2003; Szpila, 2010; Byrd and Castner, 2010; Gennard, 2010; Martín-Vega *et al.*, 2014; Grzywacz *et al.*, 2015; Lutz *et al.*, 2018). Depending on the collector, and life stage of the insects, some of the entomological specimens were killed by direct placement in formalin, ethanol, and water (including near boiling water). Some of the entomological

specimens terminated by direct placement in near boiling water were stored in ethanol. In some cases, some of the immature entomological specimens collected were reared to adulthood in the Forensic Entomology Laboratory at the University of Cape Town's Health Sciences Campus for accurate species identification.

2.10. Statistical Analysis

The data generated during the course of this study were captured and stored in Microsoft Excel. All statistical analysis was conducted in the R Statistical Environment (R Core Team 2023) with significance set *a priori* at $\alpha = 0.05$. Family-wise error rates were accounted for in post-hoc tests using Tukey's Honest Significant Difference (HSD) test or Dunn's test functions.

2.10.1. Insect data

In each of the neonate and adult pig trials, the number of insect species and individuals found on each carcass and in each decomposition stage was counted, recorded, and used for the analysis. The number of insect species refers to those identified to the species level. For some insect families (e.g., Dermestidae) it was not possible to identify to the species level, and these were grouped as one "species" (i.e., Dermestidae gen. sp.). Secondly, some insect individuals belonging to the same family but morphologically distinctive, were regarded as a single individual "species" (e.g., Muscidae gen. sp. 1, Muscidae gen. sp. 2, Muscidae gen. sp. 3 and so on). Thirdly, members of some insect orders (e.g., Blattodea) not identified to the family, genus, or species level were grouped as one "species" (i.e., Blattodea fam. gen. sp.), following the methodology of Sigovini *et al.* (2016).

In each of the neonate pig trials, the number of insect species and individuals attracted to the two pig carcasses were pooled together and then used for the analysis. The number of insect species and individuals (response variables) at different stages of decomposition (explanatory variable) were analyzed with a Generalized Linear Model (GLM) and the Poisson distribution was assumed (for count data). Similarly, the GLM was used to explain the relationship between the number of insect species and individuals in relation to the stages of decomposition, sampling trials and the interactions between these variables. Following the GLM analyses, the best model was selected using the Akaike's Information Criterion (AIC).

The significance of the GLM model was tested using the *Anova* function in the *car* package. Where the GLM model was significant, pairwise comparisons were carried out using the Tukey's HSD test function in the *agricolae* package. To avoid ambiguity, the abundance, and successional patterns of adult insects attracted to each of the neonate and adult pig

carcasses were presented using separate occurrence matrices as suggested by Michaud *et al.* (2012) and Matuszewski (2021) and these matrices were generated using a combination of the *tidyverse* and *ggplot2* packages.

2.10.2. Influence of carcass size on insect species and individuals

To examine if the size of the carcass had an influence on the number of insect species and individuals attracted to the decomposing pig carcasses, data relating to the number of insect species and individuals generated from each of the neonate pig trials across the summer (i.e., early, mid-, and late summer) and winter (i.e., early, mid-, and late winter) seasons were individually compared to similar data generated in the adult pig summer and winter seasonal trials respectively. The number of insect species and individuals were designated as the response variables while carcass size (i.e., neonate and adult pig) was designated as the explanatory variable. The GLM with Poisson distribution was used to analyze this data. The *Anova* function was used to test the significance of the GLM model. Where the GLM model was significant, pairwise comparisons were carried out using the Tukey's HSD test function.

2.10.3. Soil pH and electrical conductivity

In each of the neonate and adult pig trials, the soil pH and electrical conductivity data were assessed to meet the assumptions of homogeneity of variances and normality using the Levene's test and Shapiro-Wilk test, respectively. All the data did not meet the assumptions for a parametric test. Consequently, differences between pH and electrical conductivity of soil samples were assessed using the Wilcoxon signed-rank test. The soil pH and electrical conductivity readings were designated as the response variables and the sources (i.e., carcass and control) were designated as the explanatory variables. To examine the influence of decomposition stage on the pH and electrical conductivity of the carcass soil, the carcass soil pH and electrical conductivity were designated as the response variables while the stages of decomposition were designated as the explanatory variables.

Differences in the carcass soil pH and electrical conductivity across the decomposition stages of the pig carcasses were assessed using the one-way analysis of variance (ANOVA) or Kruskal-Wallis rank sum tests after testing for homogeneity of variances and normality. Where significant differences were identified, post-hoc tests were carried out using the Tukey's HSD test function or Dunn's test function (with Bonferroni p-value adjustment) in the *FSA* package. Daily changes in the soil pH and electrical conductivity of the carcass and control soil were reported as means $\pm$ standard deviation (SD).

To examine whether the size of the carcass had an influence on changes in the pH and electrical conductivity of the underlying soil, the pH and electrical conductivity data of the carcass and control soils generated from each of the neonate pig trials across the summer (i.e., early, mid-, and late summer) and winter (i.e., early, mid-, and late winter) seasonal periods were compared to similar data generated in the adult pig summer and winter seasonal trials respectively. Differences between the pH and electrical conductivity of soils underneath the neonate and adult pig carcasses and those of the control soils were assessed using the Welch Two Sample t-test and Wilcoxon signed-rank test for parametric and non-parametric data respectively. The carcass and control soil pH and electrical conductivity data were designated as the response variables while carcass size was designated as the explanatory variable.

2.10.4. Carcass skin pH

Differences in carcass skin pH across the various stages of decomposition of the neonate and adult pig carcasses were examined. Here, the carcass skin pH was designated as the response variable while the stages of decomposition were designated as the explanatory variables. Following homogeneity of variances and normality assumptions verification, differences in the carcass skin pH across the decomposition stages of the pig carcasses were assessed using the one-way analysis of variance (ANOVA) or Kruskal-Wallis rank sum tests. Where significant differences exist, post-hoc tests were carried out using the Tukey's HSD or Dunn's tests. Daily changes in the carcass skin pH were reported as means $\pm$ standard deviation (SD).

To examine whether the size of the carcass has an influence on changes in the pH of the carcass skin, carcass skin pH data generated from each of the neonate pig trials across the summer (i.e., early, mid-, and late summer) and winter (i.e., early, mid-, and late winter) seasonal periods were compared to similar data generated in the adult pig summer and winter seasonal trials respectively. All the data failed to meet the requirements for a parametric test. Thus, differences in carcass skin pH between the neonate and adult pig carcasses were assessed using the Wilcoxon signed-rank test. The carcass skin pH data was designated as the response variable while carcass size was designated as the explanatory variable.

2.10.5. Relationship between soil pH/EC, ADD, RH, and RF

The time since carcass deposition was expressed as ADD. Prior to the commencement of the statistical modelling, the control soil pH and electrical conductivity data were subtracted from those of the carcass soil in all the neonate pig trials (Aitkenhead *et al.*, 2015). The values obtained were subsequently designated as Δ pH and Δ EC indicating the changes in soil pH and

electrical conductivity respectively. Thereafter, all ΔpH , ΔEC , ADD, ambient relative humidity and rainfall data from the neonate pig trials conducted within the warm (from December to May) and cold (from June to November) seasonal periods for this region were respectively pooled together to generate two distinct datasets for the warm and cold seasons.

Owing to the lack of linear relationships after examining the scatter plot between $\Delta\text{pH}/\Delta\text{EC}$ and weather variables, the Generalized Additive Model (GAM) was used to evaluate the impact of ADD, ambient relative humidity and rainfall on $\Delta\text{pH}/\Delta\text{EC}$ for the warm and cold seasonal periods. To perform the GAM analyses, the *gam* function from the *mgcv* package was used to fit the models. ΔpH and ΔEC were respectively modelled as a function of ADD, ambient relative humidity and rainfall. Furthermore, ADD, ambient relative humidity and rainfall were included as smooth terms in the GAM models.

2.10.6. Relationship between carcass skin pH, ADD, RH, and RF

All carcass skin pH, ADD, ambient relative humidity and rainfall data from the neonate pig trials conducted within the same seasonal periods mentioned above were respectively pooled together to generate two datasets for the warm and cold seasons. GAM was used to evaluate the impact of ADD, ambient relative humidity and rainfall on carcass skin pH for the warm and cold seasonal periods. The carcass skin was modelled as a function of ADD, ambient relative humidity and rainfall. In the GAM models, the ADD, ambient relative humidity and rainfall were included as smooth terms.

2.10.7. Retrospective review

Data were analyzed descriptively. Taxonomic order was assessed at both the species and family level. Where insects were not identified to the species level, these were grouped and treated as separate "species" (i.e., Dermestidae gen. sp., Cleridae gen. sp.) for analysis.

2.11. Ethical considerations

2.11.1. Neonate and adult pig decomposition trials

Ethical clearance for the use of the neonate and adult pig carcasses, and the termination protocol alongside the sampling, rearing and termination of collected insects was obtained from the University of Cape Town's Faculty of Health Sciences Animal Ethics Committee (UCT_FHS AEC: 019_036 and AEC: 021_021; Figures A1 – A4).

2.11.2. Review

Ethical approval to access entomological reports associated with human case reports in the Forensic Pathological Service Autopsy Office Database of the Division of Forensic Medicine

and Toxicology was obtained from the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee (UCT_FHS HREC: 528_2021; Figures A5 & A6). Furthermore, permission to access other entomological reports was obtained from the founding Directors (Drs Marise Heyns and Devin Finaughty) of CapeFORTE.

2.11.3. Table Mountain National Park

Permission to conduct the study in the Table Mountain National Park was obtained from the authorities of the South African National Park/Table Mountain National Park (Permit numbers: CRC/2020-2021/024—2019/V1; CRC/2021/024--2019/V1; CRC/2022-2023/024--2019/V1; Figures A7 – A12).

Chapter 3: Results and Discussion – Neonate Pig Decomposition and Insect Succession Trials

3.1. Spring Season

3.1.1. Weather data

Early spring

In this trial, daily mean internal and external carcass temperatures ranged between 10.9°C to 21.4°C (overall mean = 15.9°C) and 11.1°C to 20.8°C (overall mean = 15.9°C) respectively (Figure 3.1A). Daily mean ambient temperature ranged between 10.8°C to 20.2°C (overall mean = 15°C) (Figure 3.1A). Daily mean ambient relative humidity was between 47.8% to 90.9% (overall mean = 71.3%) while daily rainfall was between 0.0mm to 25.3mm (overall mean = 2.48mm) (Figure 3.2A).

Mid-spring

In this trial, daily mean internal and external carcass temperatures ranged between 13.8°C to 22.9°C (overall mean = 18.4°C) and 14°C to 23.9°C (overall mean = 18.4°C) respectively (Figure 3.1B). Daily mean ambient temperature ranged between 13.3°C to 22.6°C (overall mean = 17°C) (Figure 3.1B). Daily mean ambient relative humidity was between 59.5% to 93.3% (overall mean = 74.5%) while daily rainfall was between 0.0mm to 48mm (overall mean = 5.04mm) (Figure 3.2B).

Late spring

In this trial, daily mean internal and external carcass temperatures ranged between 17.2°C to 24.2°C (overall mean = 21.3°C) and 15.9°C to 23.9°C (overall mean = 20.5°C) respectively (Figure 3.1C). Daily mean ambient temperature ranged between 14.6°C to 23.3°C (overall mean = 17.7°C) (Figure 3.1C). Daily mean ambient relative humidity was between 48.1% to 80.6% (overall mean = 69.2%) while daily rainfall was between 0.0mm to 18mm (overall mean = 1.31mm) (Figure 3.2C).

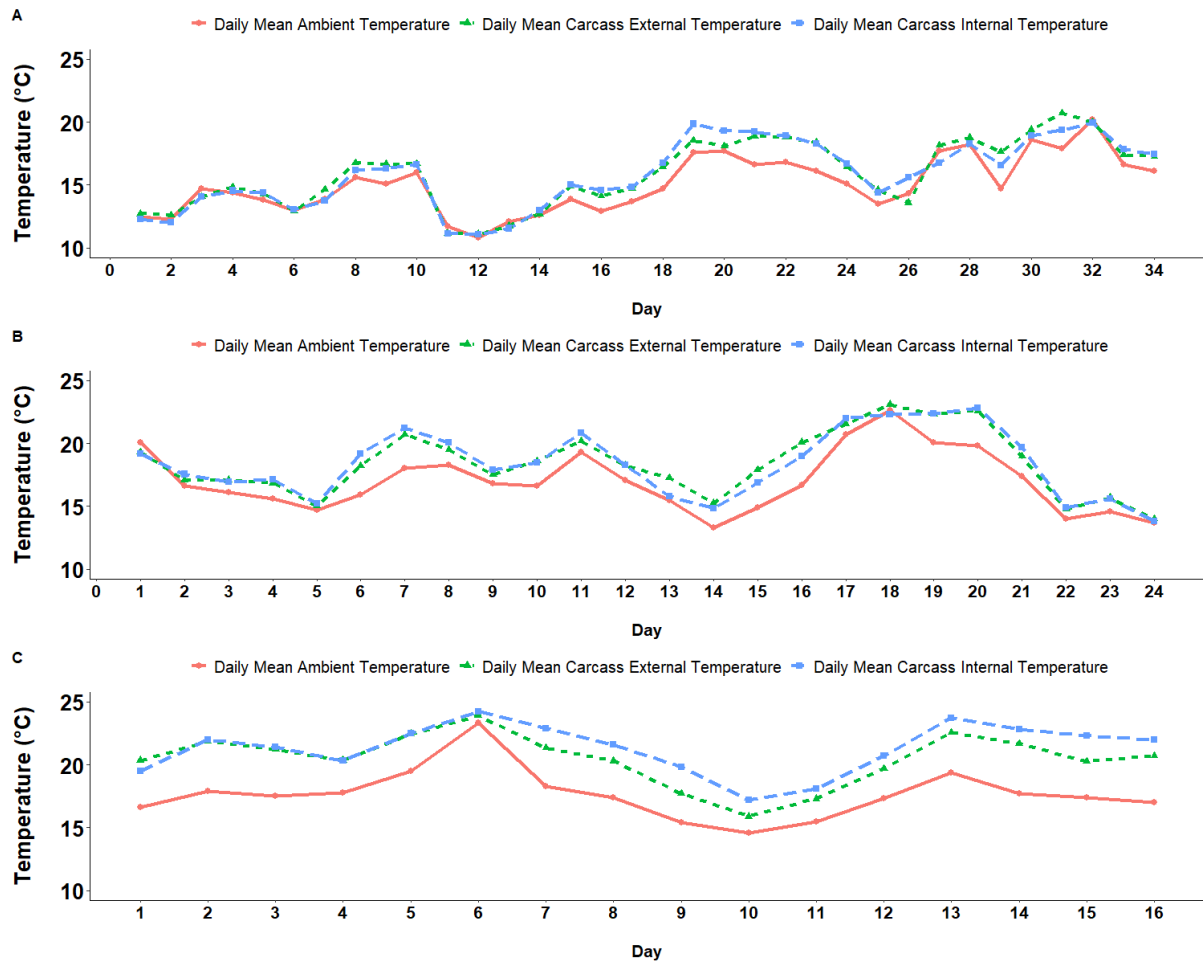


Figure 3.1: Daily mean ambient, external, and internal carcass temperatures in the early (A), mid- (B), and late spring (C) neonate pig trials.

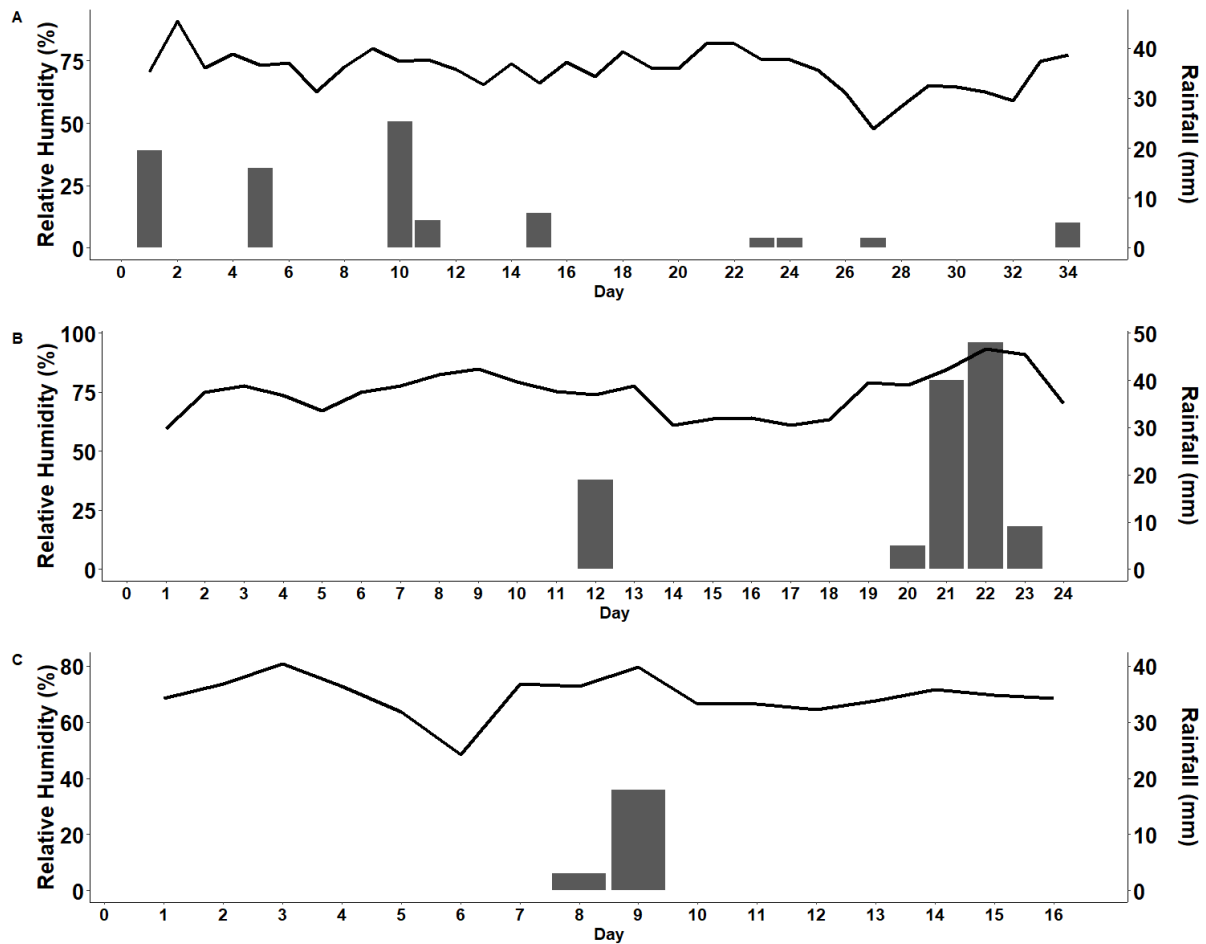


Figure 3.2: Daily mean ambient relative humidity (line) and rainfall (bars) during the early (A), mid- (B), and late spring (C) neonate pig trials.

3.1.2. Decomposition data

Fresh Stage (TBS = 3): This stage began following the complete thawing and deployment of the carcasses, and it was characterized by little or no changes in coloration and appearance of the carcasses (Figures 3.3A & 3.3E, B1A & B1E, and B2A & B2E). This stage lasted for only one day in the early spring (ADD = 12.5), mid-spring (ADD = 19.3), and late spring (ADD = 20.1) trials.

Early Decomposition Stage (TBS: 4 – 16): This stage began with a change in the coloration and appearance of the carcasses. The carcasses had a characteristic glossy appearance, and this was followed by the swelling of the neck, abdomen, and limbs, and at the same time, presence of adult and immature (i.e., larvae) insects in the oral and nasal cavities. Also, the carcasses began to show some signs of skin sloughing and hair slippage, release of decomposing fluids, and extensive maggot presence in the oral cavities and neck regions (Figures 3.3B & 3.3F, B1B

& B1F, and B2B & B2F). In the early spring trial, both carcasses transitioned into the early decomposition stage on Day 2 (ADD = 24.8), however, this stage came to an end in one of the carcasses on Day 17 (ADD = 235.6) and the other on Day 19 (ADD = 271.5). Similarly, in the mid-spring trial, both carcasses transitioned into the early decomposition stage on Day 2 (ADD = 36.6), however, the early decomposition stage came to an end in one of the carcasses on Day 7 (ADD = 125.4) and in the other on Day 13 (ADD = 236.8). In the late spring trial, the early decomposition stage in both carcasses began on Day 2 (ADD = 41.9) and ended on Day 6 (ADD = 130.1).

Advanced Decomposition Stage (TBS: 17 – 24): This stage began with the caving in of the flesh of the neck and abdominal regions of the carcasses, followed by an extensive release of cadaveric fluids from the abdomen. Also, the carcasses began to show some signs of extensive skin slippage, bone exposure and detachment in the three body scoring regions (i.e., head and neck, trunk, and limbs) (Figures 3.3C & 3.3G, B1C & B1G, and B2C & B2G). Flesh fly and blow fly larvae aggregating in the oral cavity began to move through the neck to the abdominal region where their feeding activities were mostly restricted. Some of the larvae migrated from the carcasses possibly due to the reduction in food resource or search for suitable pupariation sites. In the early spring trial, the advanced decomposition stage began in one of the carcasses on Day 18 (ADD = 252.3) up to Day 26 (ADD = 392.1), and in the other carcass on Day 20 (ADD = 290.2) up to Day 28 (ADD = 428.1). In the mid-spring trial, the duration of the advanced decomposition stage for one of the carcasses was between Day 8 (ADD = 145.2) and Day 10 (ADD = 181.5), while the other was between Day 14 (ADD = 251.8) and Day 17 (ADD = 310.6). In the late spring trial, the advanced decomposition stage began on Day 7 (ADD = 151.9) and ended on Day 12 (ADD = 244.9).

Skeletonization Stage (TBS $\geq$ 25): This stage was mainly characterized by the presence of bones, greasy substances, and dried skin or mummified tissues. At times, the dried skin takes the form of a “mat”, covering the underlying bones and greasy substances (Figures 3.3D & 3.3H, B1D & B1H, and B2D & B2H). In the early spring trial, this stage began on Day 27 (ADD = 409.6) in one of the carcasses and on Day 29 (ADD = 445.2) in the other carcass. In the mid-spring trial, the carcasses transitioned into the skeletonization stage on Day 11 (ADD = 202) and Day 18 (ADD = 333.3), respectively. In the late spring trial, both carcasses transitioned into the skeletonization stage on Day 13 (ADD = 267.8).



Figure 3.3: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the early spring neonate pig trial.

3.1.3. Abundance and diversity of identified insect taxa

Early spring

A total of 7,441 adult insect individuals belonging to five orders (Diptera, Hymenoptera, Coleoptera, Blattodea and Orthoptera) were recorded. Amongst the insect orders recorded, the order Diptera (60.3%) was the most abundant followed by the order Coleoptera (21.5%), Hymenoptera (18%), fewer individuals (<1%) of Blattodea and Orthoptera (Figure 3.4A). Within the order Diptera, a total of 4,490 individuals were recorded of which members of the family Calliphoridae (59.6%) were the most abundant followed by the families Muscidae (16.59%), Piophilidae (15.66%), Drosophilidae (4.14%), Sarcophagidae (1.76%), and fewer individuals (<1%) of the families Anthomyiidae, Heleomyzidae, Sepsidae, Scathophagidae, Sciariidae, Phoridae, Curtonotidae and Tephritidae (Figure 3.4B). Within the family Calliphoridae, a total of 2,676 Calliphorid flies were recorded of which *Chrysomya chloropyga* (Weidemann, 1818) (87.86%) was the most abundant, followed by *Ch. albiceps* (7.25%), *Calliphora croceipalpis* (Jaennicke, 1867) (2.47%), *Chrysomya megacephala* (Fabricius, 1794) (1.35%), and fewer individuals (<1%) of *Calliphora vicina* (Robineau-Desvoidy, 1830), *Chrysomya marginalis* (Robineau-Desvoidy, 1830), *L. sericata* and *Lucilia cuprina* (Weidemann, 1830) (Figure 3.4C). Amongst the order Coleoptera, a total of 1,599 individuals of which the representatives of the family Histeridae (34.9%) were the most abundant, followed closely by the Dermestidae (33.3%), Silphidae (29.1%), and fewer individuals (<1%) of the families Tenebrionidae, Trogidae, Scarabaeidae, Staphylinidae and Coccinellidae (Figure 3.4D).

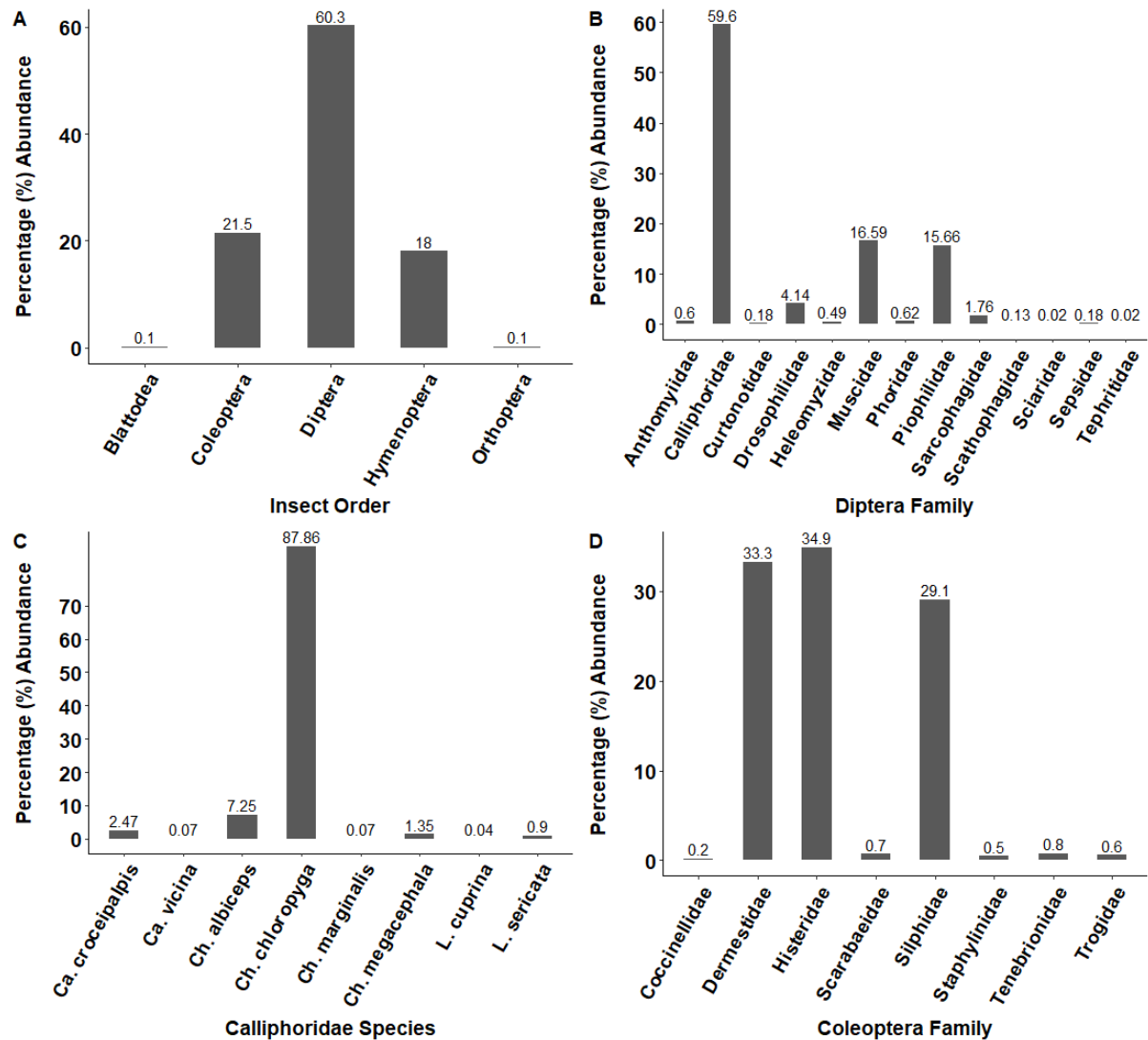


Figure 3.4: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the early spring neonate pig trial.

Mid-spring

A total of 7,993 adult insect individuals belonging to four orders (Diptera, Hymenoptera, Coleoptera, and Blattodea) were recorded. Amongst the insect orders recorded, the order Diptera (75.9%) was the most abundant followed by the order Coleoptera (15.5%), Hymenoptera (8.6%), and Blattodea (0.1%) (Figure 3.5A). Within the order Diptera, a total of 6,063 individuals were recorded of which members of the family Calliphoridae (76.56%) were the most abundant followed by the families Muscidae (9.52%), Piophilidae (8.81%), Sepsidae (2.16%), Sarcophagidae (1.8%), and fewer individuals (<1%) of the families Drosophilidae, Anthomyiidae, Scathophagidae, Phoridae, Lauxanidae, Curtonotidae and Heleomyzidae (Figure 3.5B). Within the family Calliphoridae, a total of 4,642 Calliphorid flies were recorded

of which *Ch. chloropyga* (82.27%) was the most abundant, followed by *Ch. albiceps* (11.83%), *Ch. megacephala* (4.39%), and fewer individuals (<1%) of *Ca. croceipalpis*, *Ch. marginalis*, *L. sericata*, *L. cuprina*, *Hemipyrellia fernandica* (Macquart, 1855), *Pericallimyia* sp., and *Ca. vicina* (Figure 3.5C). Amongst the order Coleoptera, a total of 1,239 individuals were recorded of which representatives of the family Dermestidae (43.3%) were the most abundant, followed closely by the Histeridae (32.4%), Silphidae (21.4%), Tenebrionidae (1.9%), and fewer individuals (<1%) of the families Staphylinidae, Coccinellidae and Trogidae (Figure 3.5D).

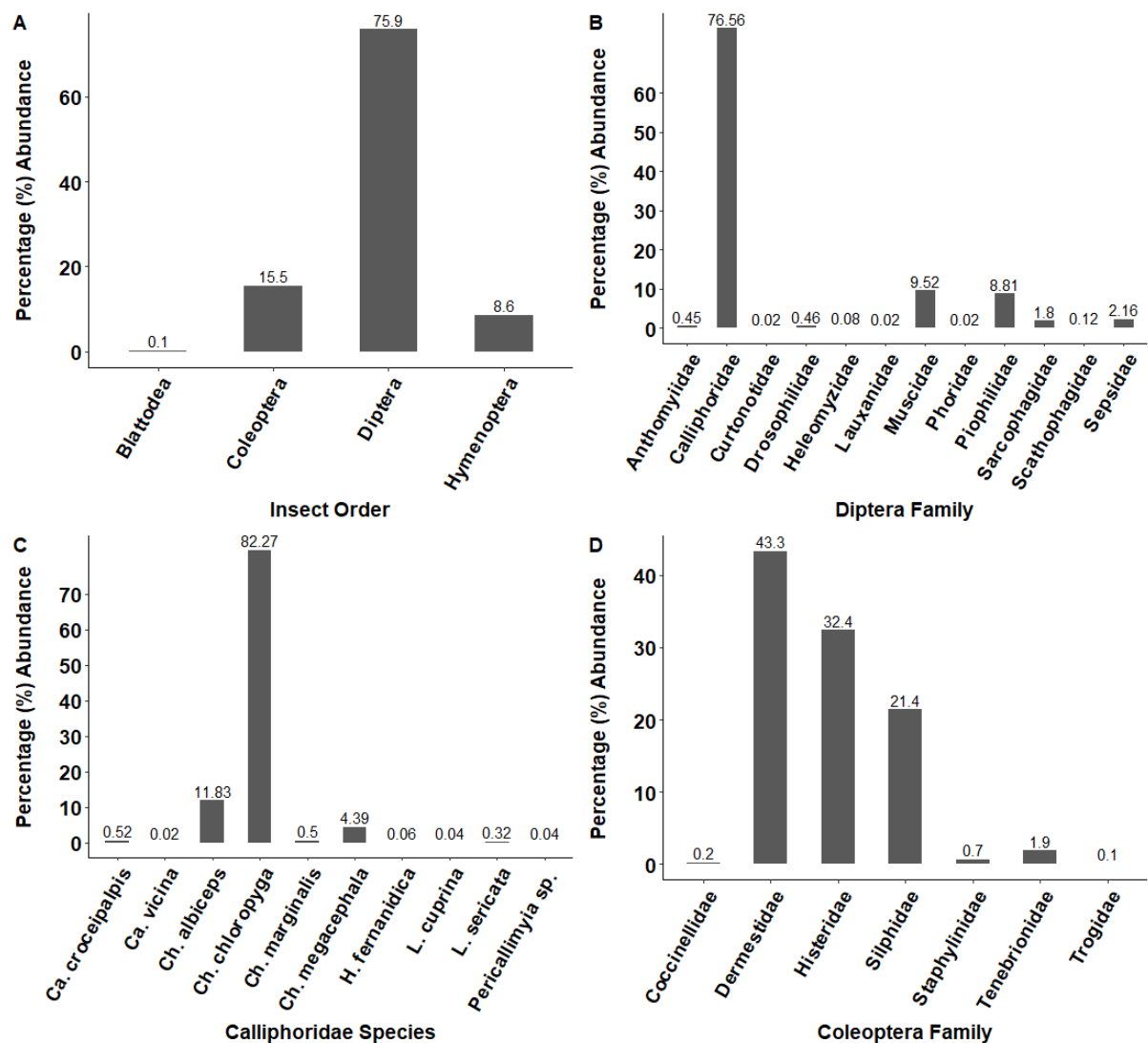


Figure 3.5: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the mid-spring neonate pig trial.

Late spring

A total of 3,140 adult insect individuals belonging to six orders (Diptera, Hymenoptera, Coleoptera, Blattodea, Hemiptera, and Odonata) were recorded. Amongst the insect orders recorded, the order Diptera (79.55%) was the most abundant followed by the order Hymenoptera (13.22%), Coleoptera (6.97%), and fewer individuals (<1%) of the orders Blattodea, Hemiptera, and Odonata (Figure 3.6A). Within the order Diptera, a total of 2,498 individuals were recorded of which members of the family Calliphoridae (55.36%) were the most abundant followed by the families Muscidae (27.5%), Piophilidae (7.73%), Sarcophagidae (6.24%), Anthomyiidae (1.28%), and fewer individuals (<1%) of the families Scathophagidae, Sepsidae, Lauxanidae, and Drosophilidae (Figure 3.6B). Within the family Calliphoridae, a total of 1,383 Calliphorid flies were recorded of which *Ch. chloropyga* (37%) was the most abundant, followed by *Ch. megacephala* (32.1%), *Ch. albiceps* (27.8%), *L. cuprina* (1%), and few individuals (<1%) of *L. sericata*, *Ca. croceipalpis*, *Ch. marginalis*, *H. fernandica*, *Pericallimyia* sp. (Figure 3.6C). Amongst the order Coleoptera, a total of 219 individuals were recorded of which representatives of the family Histeridae (42.9%) were the most abundant, followed closely by the Silphidae (24.7%), Dermestidae (20.1%), Staphylinidae (8.2%), Tenebrionidae (3.2%), and fewer individuals (<1%) of the families Scarabaeidae, and Trogidae (Figure 3.6D).

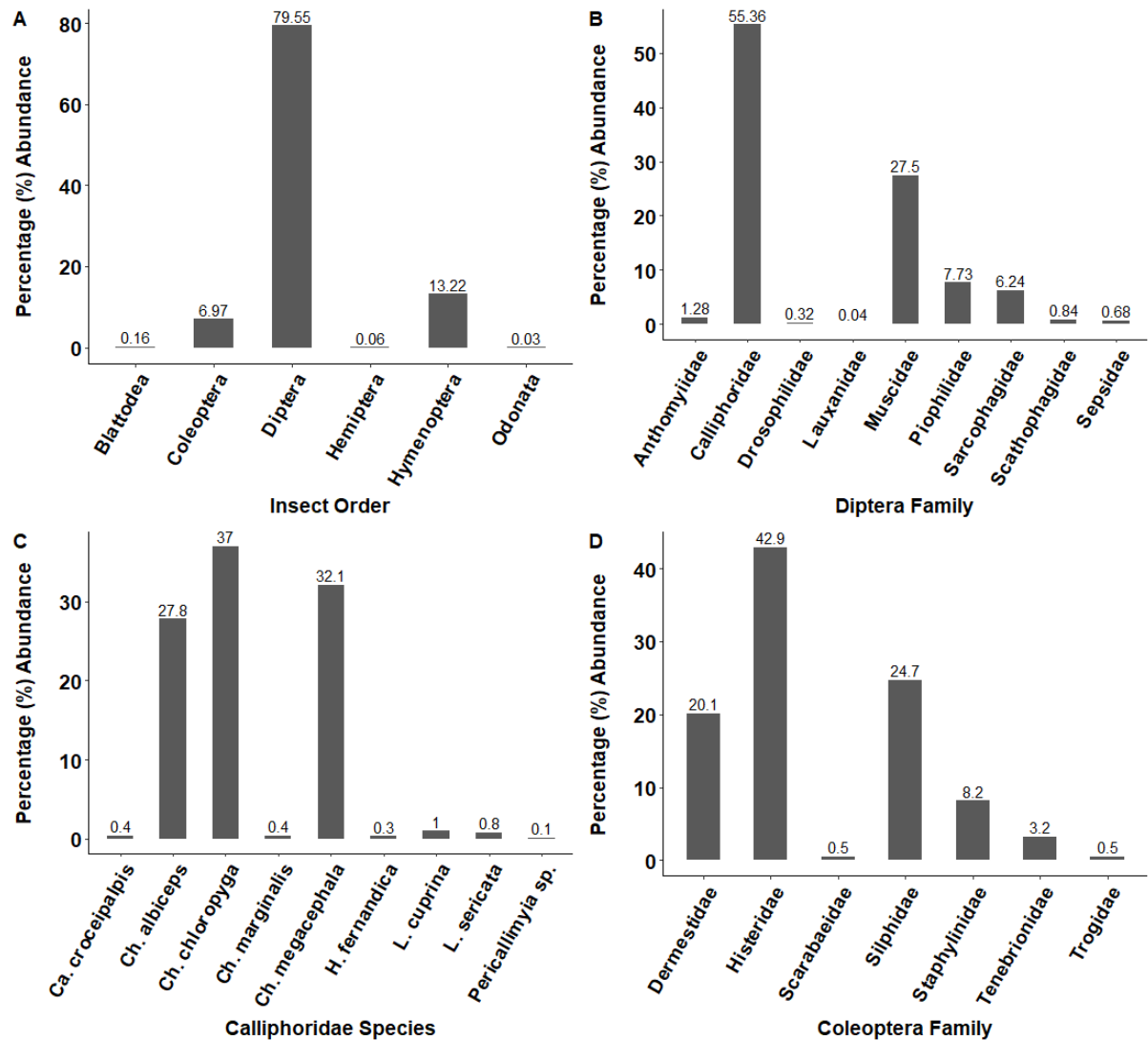


Figure 3.6: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the late spring neonate pig trial.

3.1.4. Insect diversity and abundance across various decomposition stages

In all the three experimental trials conducted in this spring study, the number of insect species significantly differed in relation to the decomposition stages (early spring: Likelihood Ratio $\chi^2 = 67.57$; DF = 3; $p < 0.0001$; mid-spring: Likelihood Ratio $\chi^2 = 64.27$; DF = 3; $p < 0.0001$; late spring: Likelihood Ratio $\chi^2 = 53.26$; DF = 3; $p < 0.0001$). Similarly, in all the three trials, there were statistically significant differences in the total number of insects with respect to the decomposition stages (early spring: Likelihood Ratio $\chi^2 = 2354.1$; DF = 3; $p < 0.0001$; mid-spring: Likelihood Ratio $\chi^2 = 2146.4$; DF = 3; $p < 0.0001$; late spring: Likelihood Ratio $\chi^2 = 730.4$; DF = 3; $p < 0.0001$). The advanced decomposition stage attracted the highest number of insect species in the early spring ($p < 0.05$) and mid-spring ($p < 0.05$) trials, while the

skeletonization stage attracted the highest number of insect species in the late spring trial ($p < 0.05$) (Figures B3A, B4A, and B5A). Similarly, the advanced decomposition stage attracted the highest number of insect individuals both in the early and mid-spring trials ($p < 0.05$) (Figures B3B & B4B). In contrast, the skeletonization stage was found to attract the highest number of insect individuals in the late spring trial ($p < 0.05$) (Figure B5B).

Insect diversity and abundance across various decomposition stages (combined data)

Combining the results obtained from the three trials within the spring season, the number of insect species differed significantly only in relation to the decomposition stages (Likelihood Ratio $\chi^2 = 67.57$; DF = 3; $p < 0.0001$). After a post-hoc Tukey test, the advanced decomposition and skeletonization stages were found to attract significantly higher numbers of insect species ($p < 0.05$) (Figure 3.7). In contrast, there was no statistically significant difference in the number of insect species with respect to the sampling trials (Likelihood Ratio $\chi^2 = 2.83$; DF = 2; $p = 0.24$). There was a statistically significant interaction in the number of insect species between the stages of decomposition and sampling trials (Likelihood Ratio $\chi^2 = 16.33$; DF = 6; $p = 0.01$). With respect to the total number of insects across the three monthly trials within the season, there was a statistically significant difference in relation to the decomposition stages (Likelihood Ratio $\chi^2 = 2354.1$; DF = 3; $p < 0.0001$). Across the four decomposition stages in all the three trials, the advanced decomposition stage attracted the highest number of insects ($p < 0.05$) (Figure 3.8A). The total number of insects differed significantly across the three sampling trials (Likelihood Ratio $\chi^2 = 41.7$; DF = 2; $p < 0.0001$). After a post-hoc Tukey test, the total number of insects in the mid-spring trial was significantly higher ($p < 0.05$) than that of the early and late spring trials (Figure 3.8B). Above all, there was a statistically significant interaction between the stages of decomposition and sampling trials (Likelihood Ratio $\chi^2 = 409.6$; DF = 6; $p < 0.0001$).

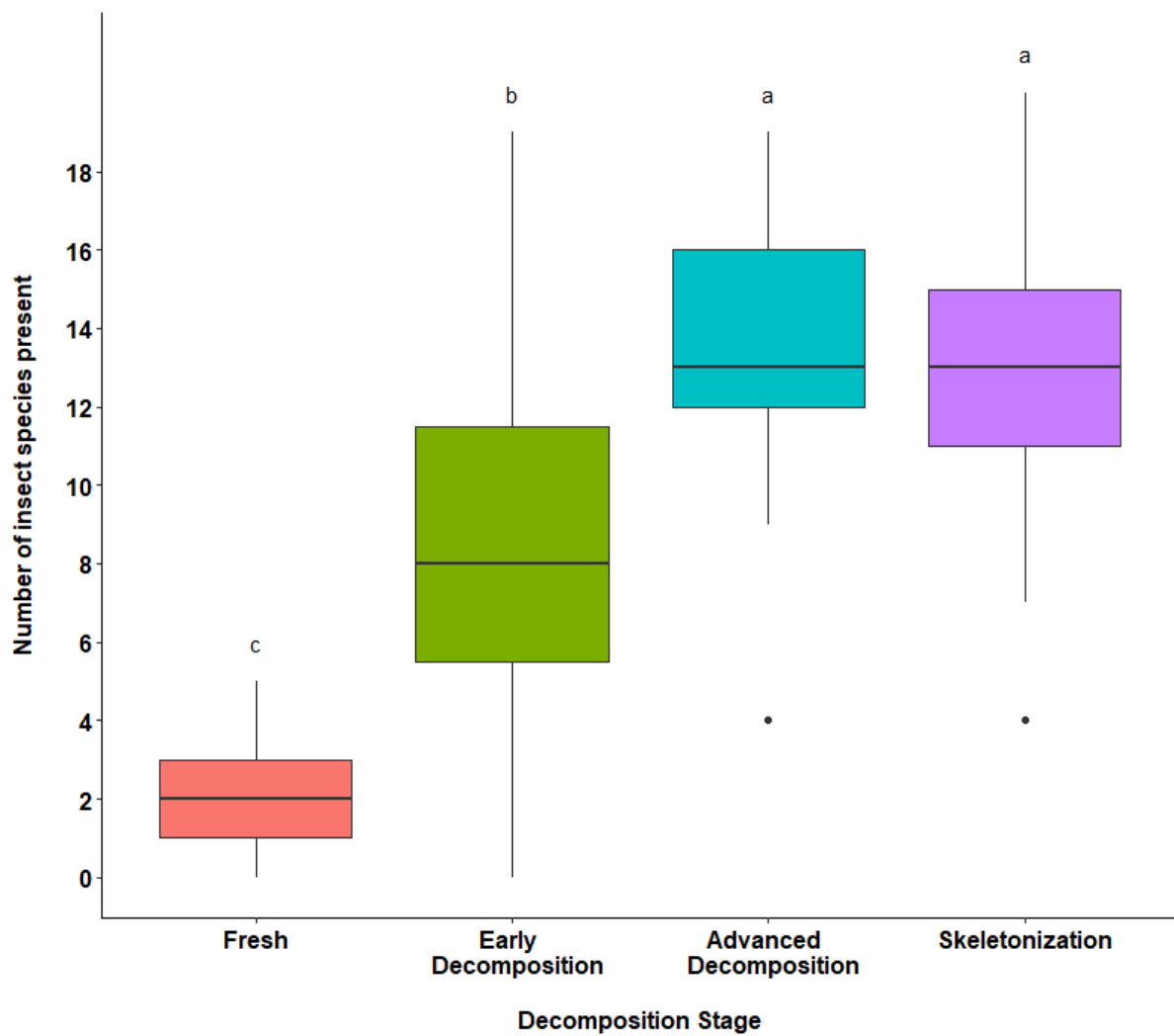


Figure 3.7: Box plot comparing the number of insect species present across the four decomposition stages in all the three neonate pig spring seasonal trials. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

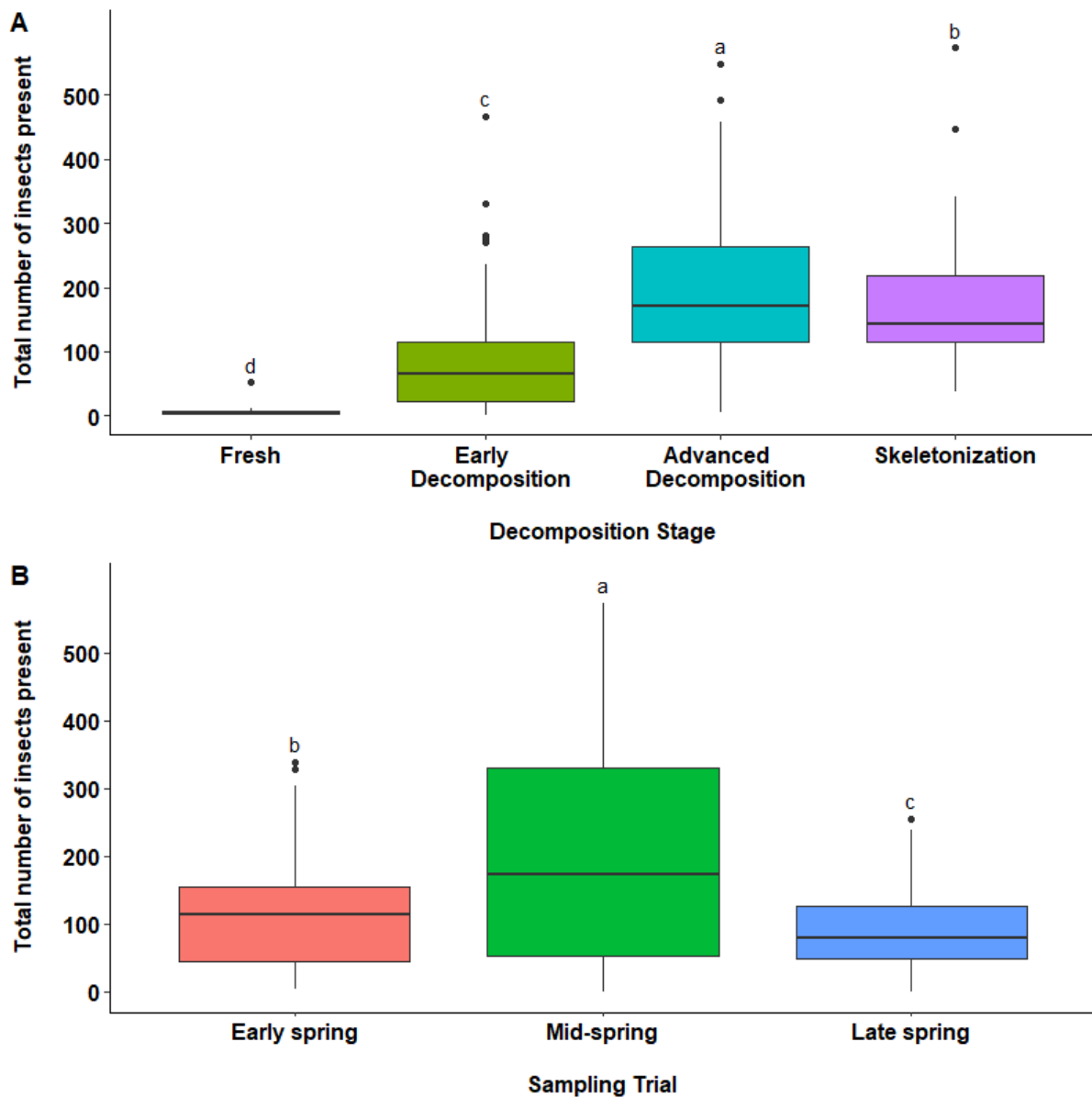


Figure 3.8: Box plots comparing the total number of insect individuals present across the four decomposition stages in all the three neonate pig spring seasonal trials (A) and total number of insect individuals present across the three neonate pig spring seasonal trials (B). Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

3.1.5. Insect succession

Diptera succession

As early as Days 1 and 2, some insect species were recorded on the carcasses across the early, mid-, and late spring trials (Figures B6 – B11). Amongst the family Calliphoridae, *L. sericata*, *L. cuprina*, *Ca. croceipalpis*, *Ch. chloropyga*, *H. fermamdica*, *Ch. marginalis*, *Ch. megacephala* and *Pericallimyia* sp. arrived on the carcasses in the fresh and early decomposition stages (Days 1 and 7) across the early, mid-, and late spring trials (Figures B6 – B11). *Calliphora vicina* arrived on the carcasses during the early decomposition stages between Days 3 and 13, but their presence was only restricted to the early and mid-spring trials (Figures B6 – B8), and entirely absent in the late spring seasonal period.

The eggs and first instar larvae of *Ca. croceipalpis* and *Ca. vicina* were recorded in the right ear, and oral cavity of the carcasses between Days 3 and 7 in the early spring seasonal period (Figures B6 & B7). The eggs and larvae of these two blow flies were not recorded on the pig carcasses in the mid-, and late spring seasonal periods. The eggs and first instar larvae of *Ch. chloropyga* were recorded in and around the ears, oral cavities, and necks of the carcasses between Days 2 and 12 specifically during the early and advanced decomposition stages across the early, mid-, and late spring trials (Figures B6, B8, B9, & B11). Following the arrival of the previously mentioned Calliphorid flies, some of them were observed to persist on the carcasses until the termination of the experimental trials across the three spring seasonal trials (Figures B6 – B11).

Across the early, mid-, and late spring trials, most members of the family Muscidae arrived on the carcasses between Days 1 and 3 (fresh and early decomposition stages), and they often persisted on carcasses until the termination of the trials (Figures B6 – B11). Muscid flies such as *Musca* sp. and *Hydrotaea* sp. were often present on the carcasses across the three spring seasonal trials between Days 3 and 9 when the carcasses were in the early and advanced decomposition stages (Figures B6 – B11). The presence of *Neomyia viridescens* (Robineau-Desvoidy, 1830) was only noticed during the mid-spring trial, specifically on Days 14 and 20 during the skeletonization stage of the carcasses (Figures B8 & B9).

Members of the family Sarcophagidae were one of the first colonizers of the pig carcasses across the early, mid-, and late spring trials, arriving between Days 1 to 8 (fresh and early phases of early decomposition) (Figures B6 – B11). The first instar larvae of this family were recorded around the limbs of the pig carcasses between Days 4 and 8 across the three

spring seasonal trials (Figures B7, B9 – B11). As with the Muscid flies, they were mostly observed to maintain a constant presence until the skeletonization stage of the pig carcasses (Figures B6 – B11).

Suillia picta (Weidemann, 1830) was only observed in the early and mid-spring seasonal trials and they were found arriving on the carcasses as early as Days 2 and 3 (early decomposition) whilst maintaining a constant presence until the skeletonization stage (Figures B6, B7, & B9). Across the three spring seasonal trials, members of the family Piophilidae, Phoridae, and Sepsidae were observed to colonize the carcasses in the late phases of early decomposition (between Days 4 to 25) and their abundance generally increased as the carcasses transition into the advanced decomposition and skeletonization stages (Figures B6 – B11). The colonization patterns of the members of other Dipteran families of little forensic importance such as Anthomyiidae, Scathophagidae, Sciariidae, Drosophilidae, Lauxanidae, Tephritidae and Curtonotidae on the pig carcasses were considerably variable, arriving during the early and advanced decomposition stages across the three spring seasonal trials (Figures B6 – B11).

Coleoptera succession

Members of the families Coccinellidae and Tenebrionidae were the first beetles arriving on the pig carcasses between Days 1 and 15 across the three spring seasonal trials (Figures B6 – B11). However, as the carcasses advanced into the late phases of early decomposition, more Coleopteran families arrived. Across the early, mid-, and late spring trials, members of the families Histeridae, Silphidae, Staphylinidae, Scarabaeidae, Dermestidae dominated the pig carcasses specifically during the advanced decomposition and skeletonization stages in varying abundances (Figures B6 – B11).

Amongst all the beetle species present, only the immature stages of Silphid and Dermestid beetles were recorded on the pig carcasses in the spring season. In the early spring seasonal period, the larvae of Silphid beetles were recorded on the carcasses during the late phase of advanced decomposition (Days 26 and 27) until the end of the trial (Figures B6 & B7). However, in the mid-spring seasonal trial, the larvae of Silphid and Dermestid beetles were recorded during the skeletonization stage of one of the carcasses (Day 22), while on the other carcass, only the larvae of Silphid beetles were observed (Day 17) (Figures B8 & B9).

Hymenoptera and other insect orders succession

Across the three spring seasonal trials, *Crematogaster* cf. *liengmei* (Formicidae) was observed to arrive on the pig carcasses between Days 1 and 3, while the carcasses were either fresh or in the early phase of early decomposition (Figures B6 – B11). This ant species was observed to persist on the carcasses until the skeletonization stage (Figures B6 – B11). In the early spring trial, *Crematogaster* cf. *liengmei* invaded one of the carcasses in large numbers (Figures 3.9A – 3.9C). Their voracious feeding activities on the carcass, especially around the limbs, abdomen, and body orifices (ears, eyes, and mouth) resulted in the creation of several bitemarks and infliction of reddish-brown lesions on several parts of the body (Figures 3.9C & 3.9D). Furthermore, their intense presence prevented blow flies and flesh flies from laying their eggs/larvae and/or establishing larval masses on the carcass. Other ant species such as *Lepisiota* sp. and *Pheidole* sp. only arrived during the mid- and late phases of early decomposition (Day 8 and Day 20 respectively) of one of the carcasses in the early spring seasonal trial (Figure B6).

Alysia manducator (Panzer, 1799) belonging to the family Braconidae only arrived on the carcasses between Days 2 and 10 across the three seasonal trials (Figures B6 – B11). Their arrival time often coincided with the occurrence of blow fly eggs/larvae on the carcasses. As with *Crematogaster* cf. *liengmei*, *A. manducator* were consistently present on the carcasses until the skeletonization stage (Figures B6 – B11). Very few individuals of other insect orders such as Blattodea, Odonata, and Orthoptera were present on the carcasses between the early decomposition and skeletonization stages across the three spring seasonal trials (Figures B6 – B11).

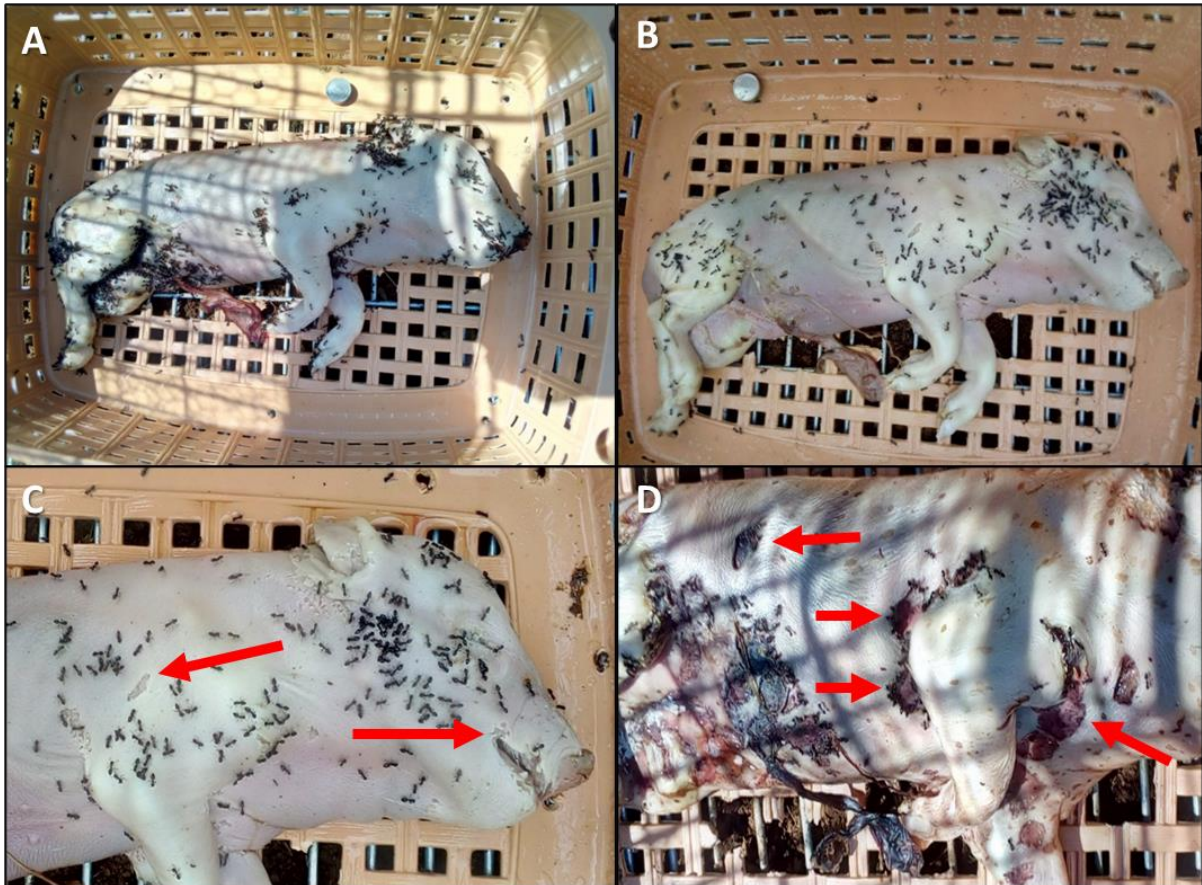


Figure 3.9: Numerous individuals of *Crematogaster* cf. *liengmei* aggregating in and around several parts of the carcass on Day 4 during the early spring trial (A). Reduced presence of *Crematogaster* cf. *liengmei* in the morning of Day 5 after a brief rainfall (B). Bitemarks and fleshy lesions (red arrows) inflicted on the carcass due to the feeding activities of *Crematogaster* cf. *liengmei* (C). Close-up view of the dry reddish-brown lesions (red arrows) inflicted on the skin of the carcass by *Crematogaster* cf. *liengmei* (Day 9) (D) (Adapted from Adetimehin *et al.*, 2022).

3.2. Summer Season

3.2.1. Weather data

Early summer

Internal and external carcass temperatures ranged between 21.4°C to 29.9°C (overall mean = 25.0°C) and 20.7°C to 27.6°C (overall mean = 24.4°C) respectively (Figure 3.10A). Daily mean ambient temperature ranged between 17.9°C to 21.8°C (overall mean = 20.6°C) (Figure 3.10A). Daily mean relative humidity was between 68.7% to 87.8% (overall mean = 74.7%), while daily rainfall was between 0.0mm to 0.2mm (overall mean = 0.04mm) (Figure 3.11A).

Mid-summer

Internal and external carcass temperatures ranged between 22.3°C to 28.2°C (overall mean = 25.0°C) and 22.1°C to 26.7°C (overall mean = 24.4°C) respectively (Figure 3.10B). Daily mean ambient temperature ranged between 19.4°C to 22.5°C (overall mean = 20.9°C) (Figure 3.10B). Daily mean relative humidity was between 60.8% to 79.8% (overall mean = 69.6%), while daily rainfall was between 0.0mm to 5.5mm (overall mean = 0.42mm) (Figure 3.11B).

Late summer

Internal and external carcass temperatures ranged between 16.8°C to 28.1°C (overall mean = 22.8°C) and 17.1°C to 28.7°C (overall mean = 22.6°C) respectively (Figure 3.10C). Daily mean ambient temperature ranged between 17.6°C to 26.1°C (overall mean = 20.5°C) (Figure 3.10C). Daily mean relative humidity was between 55.7% to 73.9% (overall mean = 68.3%), while daily rainfall was between 0.0mm to 3.0mm (overall mean = 0.25mm) (Figure 3.11C).

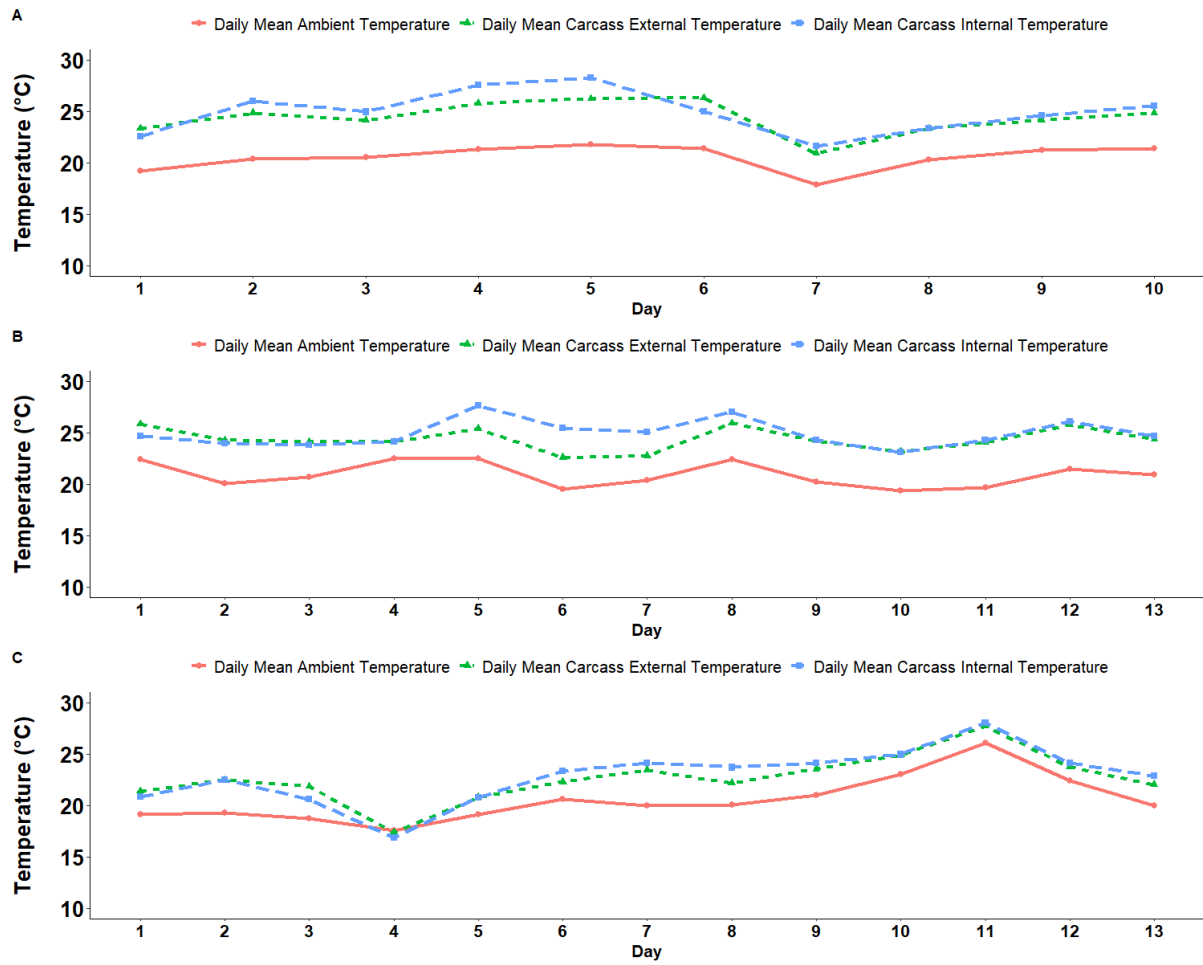


Figure 3.10: Daily mean ambient, external, and internal carcass temperatures in the early (A), mid- (B), and late summer (C) neonate pig trials.

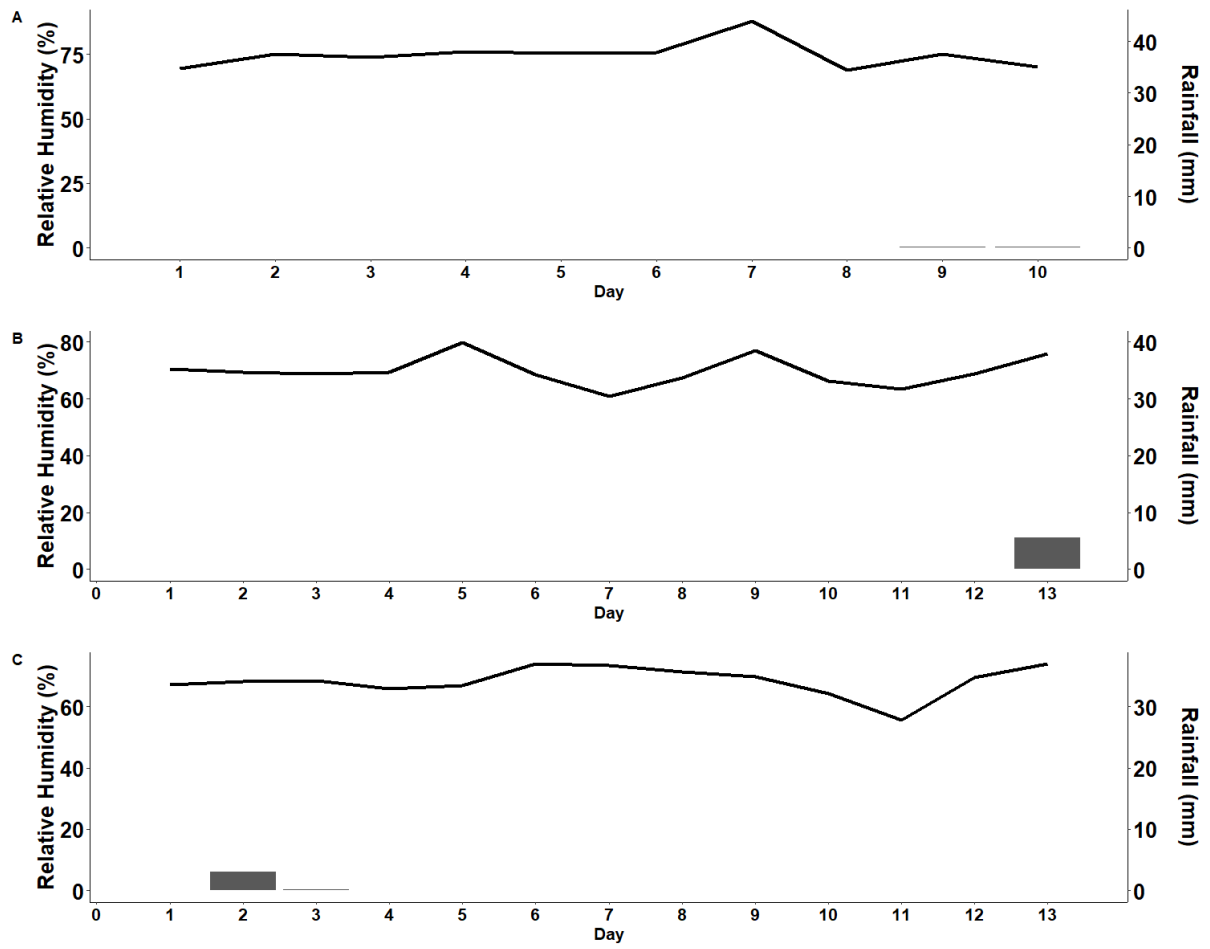


Figure 3.11: Daily mean ambient relative humidity (line) and rainfall (bars) during the early (A), mid- (B), and late summer (C) neonate pig trials.

3.2.2. Decomposition data

Fresh Stage (TBS = 3): This stage commenced immediately after the thawing and placement of the carcasses within the study site. No significant changes in the coloration of the carcasses were observed (Figures 3.12A & 3.12E, B12A & B12E, B13A & B13E). In the early and mid-summer trials, this stage lasted for period equal and/or less than 1 Day (ADD = 22.9; 25.3). However, in the late summer trial, the carcasses were fresh for a period less than 24 hours (ADD < 21.1).

Early Decomposition Stage (TBS: 4 – 16): This stage was marked by a significant change in the coloration of the head, neck, and trunk. The head and trunk became dark red in coloration. The abdominal region of the carcasses had a shiny appearance. Slight inflammation of the head, neck and abdominal region of the carcasses was noted. Furthermore, there was a significant increase in the presence and activities of both adult and immature fly species in/on the carcasses especially within the oral and nasal cavities. During the late phase of early decomposition, the carcasses began to show some signs of skin slippage and hair loss. More decomposition fluids began to seep from the abdomen into the underlying soil (Figures 3.12B & 3.12F, B12B & B12F, B13B & B13F). In the early summer trial, one of the carcasses transitioned into the early decomposition stage on Day 1 (ADD = 22.9), while the other, on Day 2 (ADD = 48.4) and this stage lasted up to Day 3 (ADD = 73) and 5 (ADD = 126.9) respectively. In the mid-summer trial, this stage commenced on Day 2 (ADD = 49.4) in both carcasses and ended on Day 6 (ADD = 148.1). In the late summer trial, this stage began as early as Day 1 (ADD = 21.1) in both carcasses and persisted up to Day 6 (ADD = 125.6).

Advanced Decomposition Stage (TBS: 17 – 24): This stage was marked by the shrinkage of the head and neck and deflation of the trunk. Huge amounts of decomposing fluids were seen oozing out from the abdominal region. Furthermore, the carcasses began to show some signs of extensive skin slippage, bone exposure and detachment in the three body scoring regions (i.e., head and neck, trunk, and limbs) (Figures 3.12C & 3.12G, B12C & B12G, B13C & B13G). Blow fly and flesh fly larvae became concentrated in the upper and lower abdominal region. During the late phase of advanced decomposition, the carcasses began to show some signs of mummification accompanied by the migration of post-feeding larvae in different directions. In the early summer trial this stage began and ended on Day 6 (ADD = 152.6) for one of the carcasses and was between Day 4 (ADD = 99.6) and 5 (ADD = 126.9) for the other carcass. In the mid- and late summer trials, the advanced decomposition stage started on Day

7 (mid-summer ADD = 172; late summer ADD = 149.4) and ended on Day 9 (mid-summer ADD = 222.7; late summer ADD = 196.1).

Skeletonization Stage ($TBS \geq 25$): This stage was characterized by the drying of the carcasses skin, and the appearance of the underlying bones' outline and greasy substances (Figures 3.12D & 3.12H, B12D & B12H, B13D & B13H). During the early summer trial, the two carcasses respectively transitioned into the skeletonization stage on Day 6 (ADD = 152.6) and 7 (ADD = 173.9). However, in the mid- and late summer trials respectively, the carcasses transitioned into the skeletonization stage on Day 10 (mid-summer ADD = 245.9; late summer ADD = 221.1).

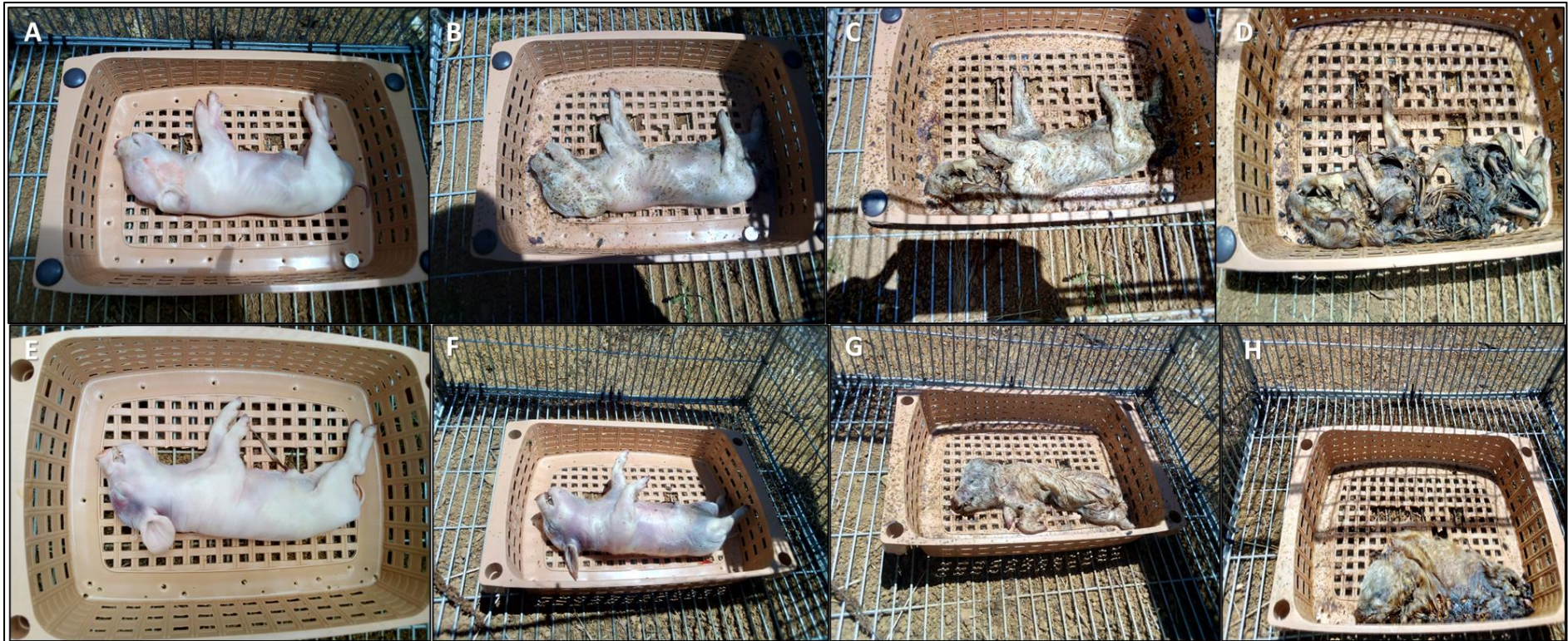


Figure 3.12: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the early summer neonate pig trial.

3.2.3. Abundance and diversity of identified insect taxa

Early summer

A total of 2,878 adult insect individuals belonging to four orders (Diptera, Hymenoptera, Coleoptera, and Hemiptera) were recorded. Amongst the insect orders recorded, the order Diptera (95%) was the most abundant followed by the orders Coleoptera (4.6%), Hymenoptera (0.3%), and Hemiptera (0.1%) (Figure 3.13A). Within the order Diptera, a total of 2,734 individuals were recorded of which members of the family Calliphoridae (83%) were the most abundant followed by the families Muscidae (10.2%), Sarcophagidae (4.4%), and fewer individuals (<1%) of the families Sepsidae, Piophilidae, Drosophilidae and Anthomyiidae, (Figure 3.13B). Within the family Calliphoridae, a total of 2,268 Calliphorid flies were recorded of which *Ch. megacephala* (58.2%), *Ch. albiceps* (27.6%), *Ch. chloropyga* (11.1%) were the most abundant, with fewer individuals (<1%) of *H. fernandica*, *Ch. marginalis*, *L. sericata* and *L. cuprina* (Figure 3.13C). Amongst the order Coleoptera, a total of 131 individuals of which the representatives of the family Dermestidae (54.2%) were the most abundant, followed closely by the Histeridae (36.6%), Staphylinidae (4.6%), Silphidae (3.1%) and fewer individuals (<1%) of the families Scarabaeidae, and Cleridae (Figure 3.13D).

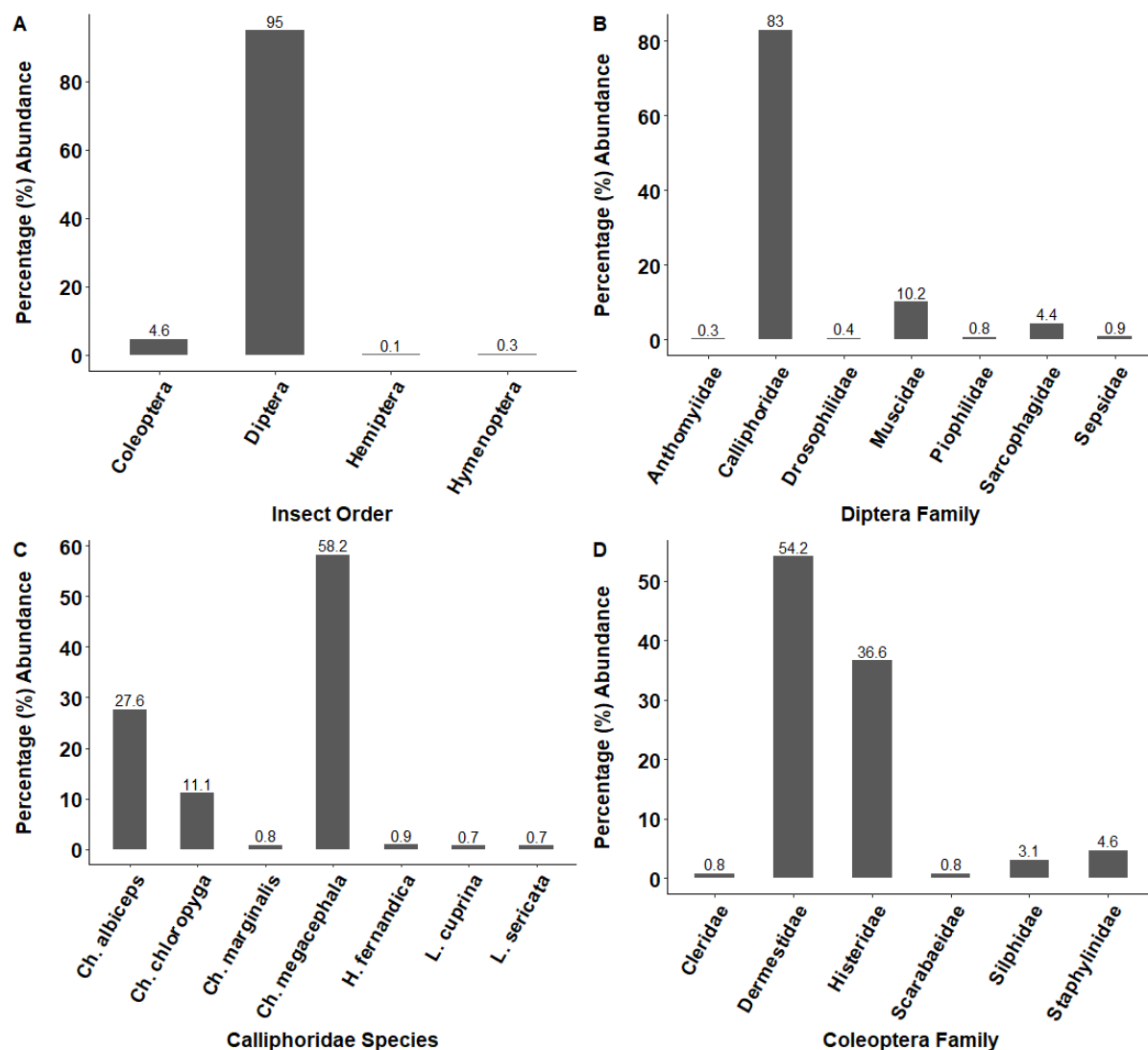


Figure 3.13: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the early summer neonate pig trial.

Mid-summer

A total of 3,679 adult insect individuals belonging to four orders (Diptera, Hymenoptera, Coleoptera, and Blattodea) were recorded. Amongst the insect orders recorded, the order Diptera (80.8%) was the most abundant followed by the orders Hymenoptera (10.8%), Coleoptera (8.3%), and Blattodea (0.1%) (Figure 3.14A). Within the order Diptera, a total of 2,973 individuals were recorded of which members of the family Calliphoridae (55.73%) were the most abundant followed by the families Muscidae (30.84%), Piophilidae (7.33%), Sarcophagidae (5.35%), and fewer individuals (<1%) of the families Sepsidae, Drosophilidae and Anthomyiidae, (Figure 3.14B). Within the family Calliphoridae, a total of 1,657 Calliphorid flies were recorded of which *Ch. megacephala* (61.6%), *Ch. albiceps* (24%), *Ch.*

chloropyga (8.7%), *L. sericata* (2.2%), *L. cuprina* (2.2%) were the most abundant, with fewer individuals (<1%) of *H. fernandica*, *Ch. marginalis*, and *Ca. croceipalpis* (Figure 3.14C). Amongst the order Coleoptera, a total of 305 individuals of which the representatives of the family Histeridae (50.5%) were the most abundant, followed by the Dermestidae (37.4%), Staphylinidae (6.2%), Silphidae (4.9%) and fewer individuals (<1%) of the families Tenebrionidae, Scarabaeidae, and Cleridae (Figure 3.14D).

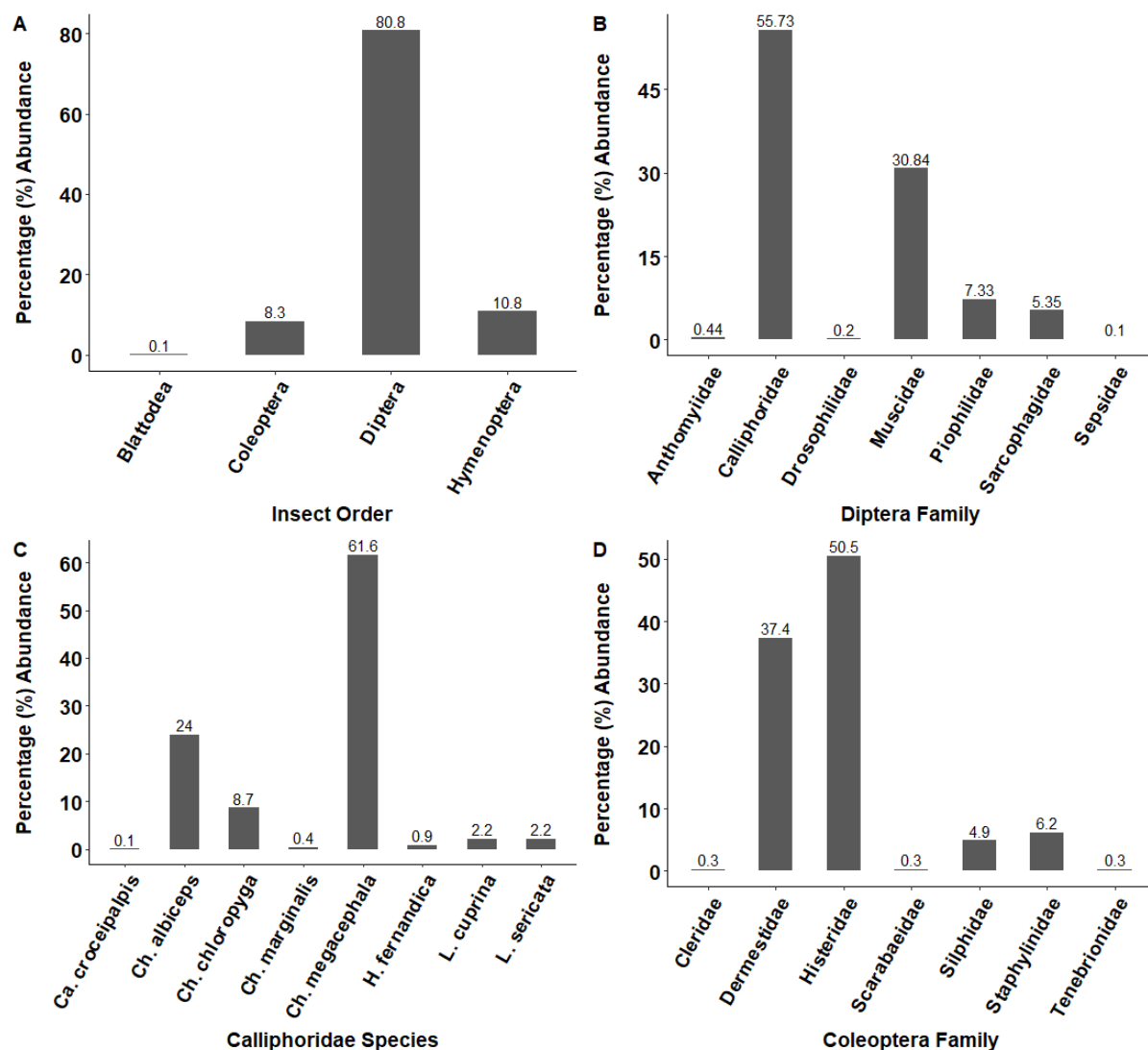


Figure 3.14: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the mid-summer neonate pig trial.

Late summer

A total of 3,152 adult insect individuals belonging to three orders (Diptera, Coleoptera and Hymenoptera) were recorded. Amongst the insect orders recorded, the order Diptera (84%) was the most abundant followed by the orders Coleoptera (12%), and Hymenoptera (4%) (Figure 3.15A). Within the order Diptera, a total of 2,662 individuals were recorded of which members of the family Calliphoridae (45.15%) were the most abundant followed by the family Muscidae (28.51%), Drosophilidae (12.92%), Sarcophagidae (10.26%), Piophilidae (1.88%), and fewer individuals (<1%) of the families Sepsidae, Sciomyzidae, and Anthomyiidae, (Figure 3.15B). Within the family Calliphoridae, a total of 1,202 Calliphorid flies were recorded of which *Ch. megacephala* (41.5%), *Ch. albiceps* (36.9%), *H. fernandica* (12.1%), *L. cuprina* (3.6%), *Ch. chloropyga* (2.8%), *Ch. marginalis* (2.3%) were the most abundant, with fewer individuals (<1%) of *L. sericata* and *Ca. croceipalpis* (Figure 3.15C). Amongst the order Coleoptera, a total of 365 individuals of which the members of the family Dermestidae (41.9%) were the most abundant, followed by the Histeridae (37.5%), Silphidae (15.1%), Staphylinidae (4.9%) and fewer individuals (<1%) of the families Tenebrionidae, and Cleridae (Figure 3.15D).

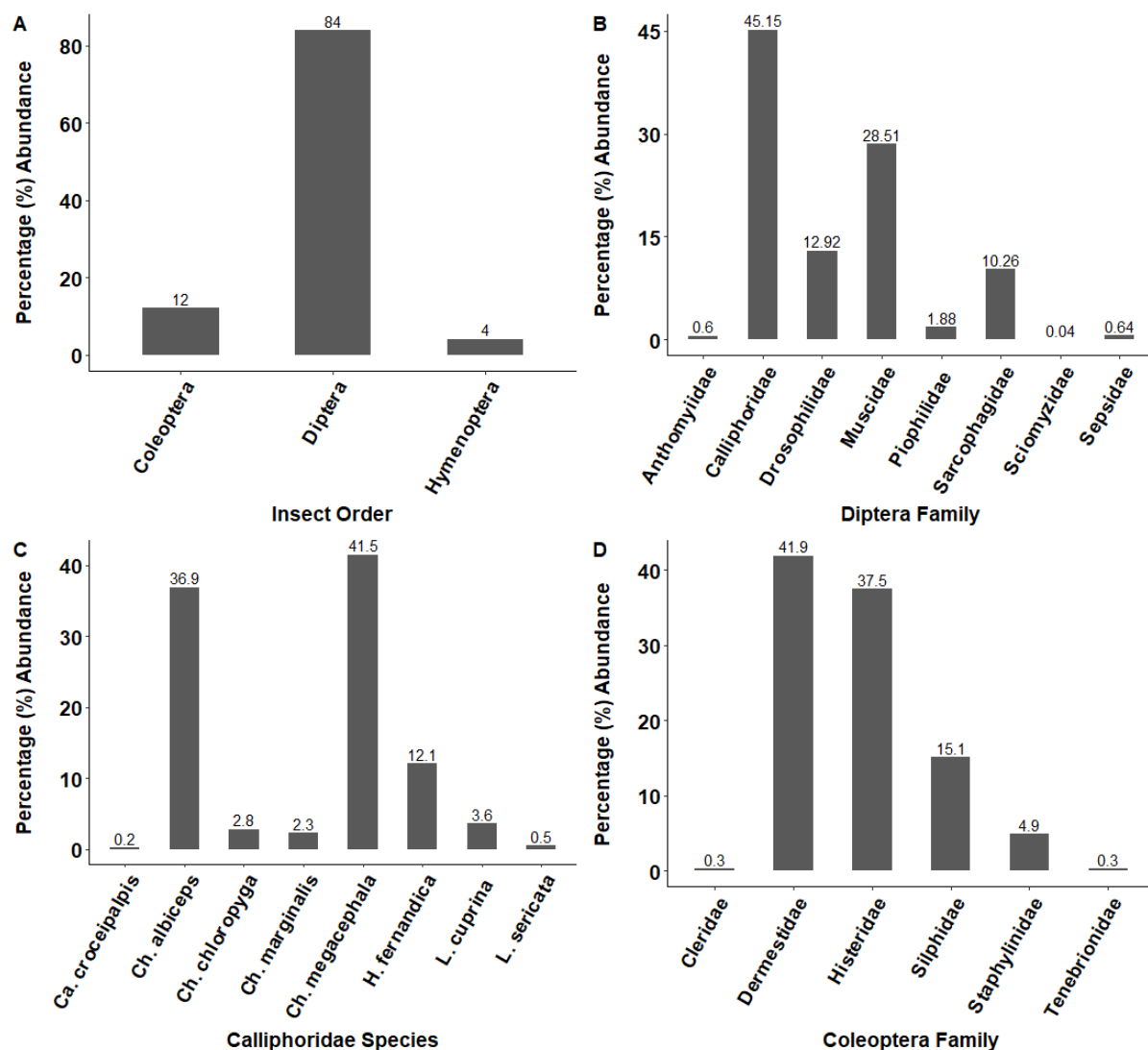


Figure 3.15: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the late summer neonate pig trial.

3.2.4. Insect diversity and abundance across various decomposition stages

In all the three trials conducted in this summer study, there was a statistically significant difference in the number of insect species in relation to the decomposition stages (early summer: Likelihood Ratio $\chi^2 = 13.95$; DF = 3; $p = 0.003$; mid-summer: Likelihood Ratio $\chi^2 = 20.49$; DF = 3; $p < 0.001$; late summer: Likelihood Ratio $\chi^2 = 12.3$; DF = 3; $p = 0.006$). Similarly, the total number of insect individuals in all the three trials were significantly different with respect to the decomposition stages (early summer: Likelihood Ratio $\chi^2 = 1372.2$; DF = 3; $p < 0.0001$; mid-summer: Likelihood Ratio $\chi^2 = 439.3$; DF = 3; $p < 0.0001$; late summer: Likelihood Ratio $\chi^2 = 473.9$; DF = 3; $p < 0.0001$). In the early, mid-, and late summer trials, the advanced decomposition stage attracted the highest number of insect species ($p < 0.05$).

(Figures B14A, B15A, and B16A) and individuals ($p < 0.05$) (Figures B14B, B15B, and B16B).

Insect diversity and abundance across various decomposition stages (combined data)

Combining the results obtained from the three trials within the summer season, the number of insect species differed significantly only in relation to the decomposition stages (Likelihood Ratio $\chi^2 = 42.84$; DF = 3; $p < 0.0001$). After a post-hoc Tukey test, the advanced decomposition and skeletonization stages were found to attract significantly higher numbers of insect species ($p < 0.05$) when compared to the fresh and early decomposition stages (Figure 3.16). In contrast, there was no statistically significant difference in the number of insect species with respect to the sampling trials (Likelihood Ratio $\chi^2 = 4.03$; DF = 2; $p = 0.13$). With respect to the total number of insects across the three monthly trials within the season, there was a statistically significant difference in relation to the decomposition stages (Likelihood Ratio $\chi^2 = 1372.2$; DF = 3; $p < 0.0001$). Across the four decomposition stages in all the three trials, the advanced decomposition attracted the highest number of insects ($p < 0.05$) (Figure 3.17A). The total number of insect individuals differed significantly across the three sampling trials (Likelihood Ratio $\chi^2 = 94.9$; DF = 2; $p < 0.0001$). After a post-hoc Tukey test, the total number of insects in the early summer trial was significantly higher ($p < 0.05$) than that of the mid- and late summer trials (Figure 3.17B). Above all, there was a statistically significant interaction between the stages of decomposition and sampling trials (Likelihood Ratio $\chi^2 = 1028.6$; DF = 6; $p < 0.0001$).

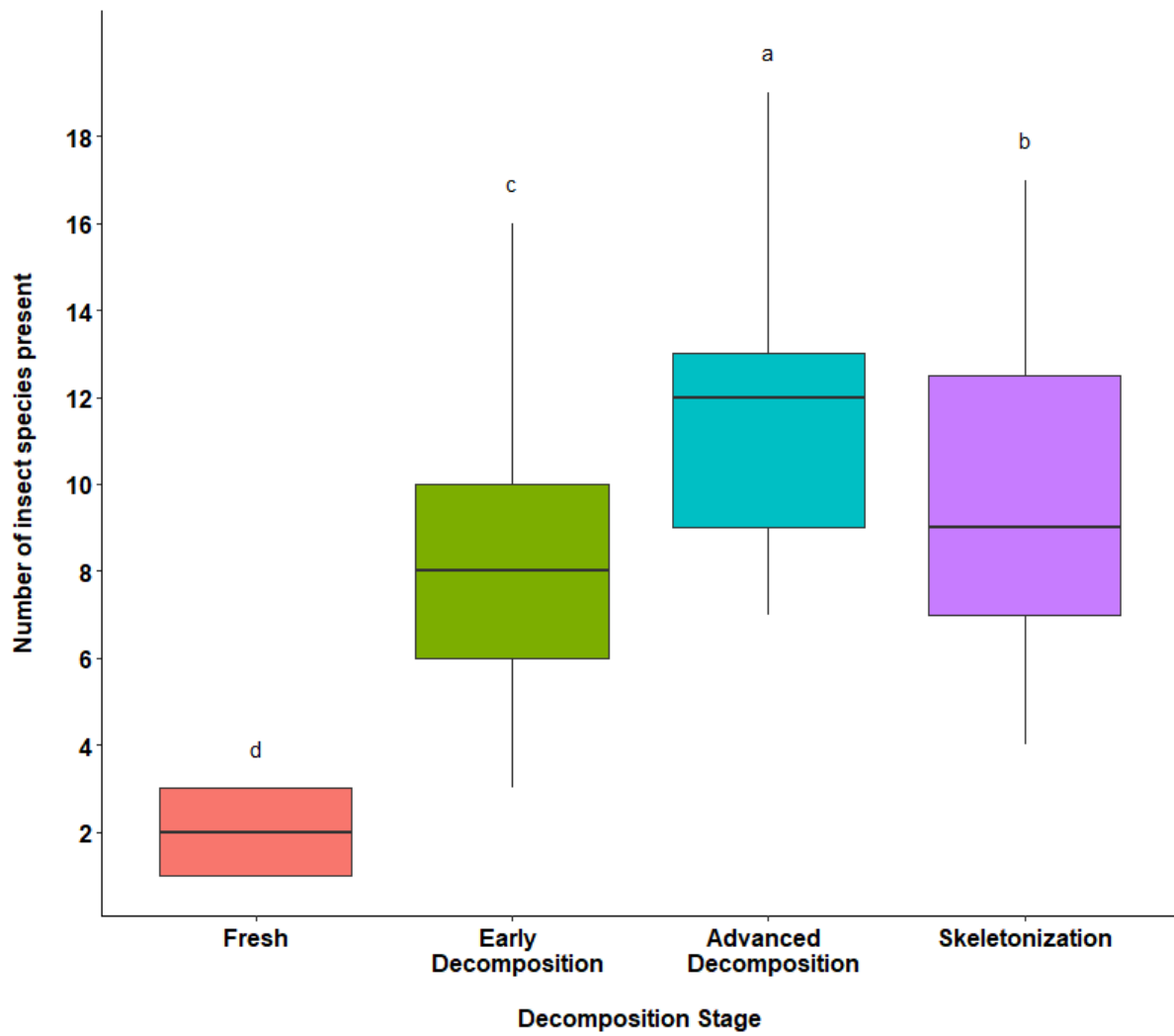


Figure 3.16: Box plot comparing the number of insect species present across the four decomposition stages in all the three neonate pig summer seasonal trials. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

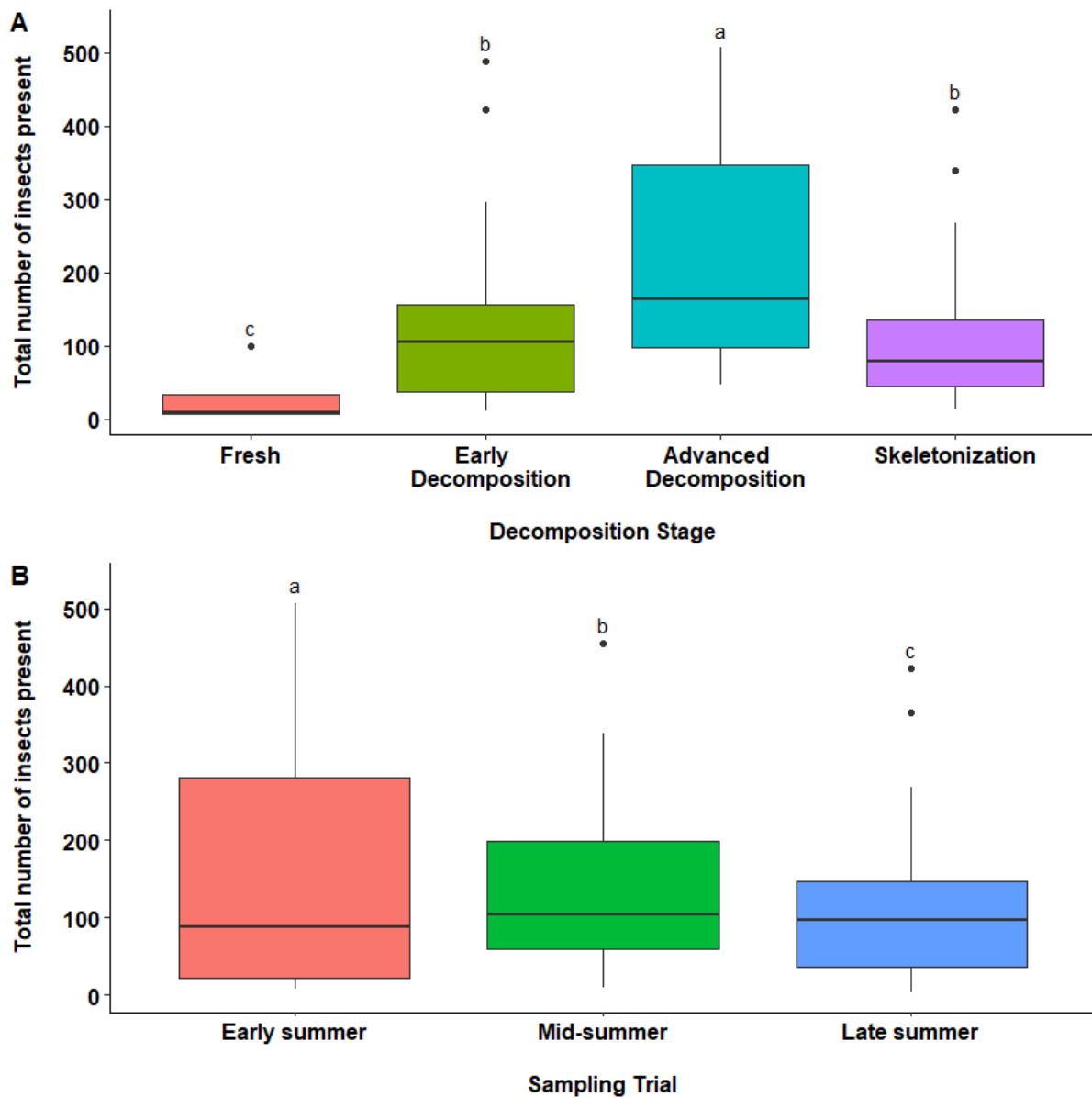


Figure 3.17: Box plots comparing the total number of insect individuals present across the four decomposition stages in all the three neonate pig summer seasonal trials (A) and total number of insect individuals present across the three summer trials (B). Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

3.2.5. Insect succession

Diptera succession

Within the family Calliphoridae, *L. sericata*, *Ch. megacephala*, *Ch. albiceps*, *Ch. chloropyga*, *Ch. marginalis*, *H. fernandica*, *L. cuprina* and *Ca. croceipalpis* all arrived on the carcasses across the early, mid-, and late summer trials between Days 1 and 4 (Figures B17 – B22). The presence of these blow flies on the pig carcasses varied across the three summer trials, with some species persisting until the termination of the field trials (Figures B17 – B22). The eggs and first instar larvae of *Ch. chloropyga*, and *Ch. albiceps* were recorded specifically around the limbs and abdominal areas of the pig carcasses between Days 4 and 6 (early and advanced decomposition stages) during the early and mid-summer trials (Figures B17 & B19). Furthermore, the eggs of *Ch. chloropyga* were recorded in the oral cavity of one of the pig carcasses on Day 8 (advanced decomposition stage) during the late summer trial (Figures B21).

The first instar larvae of *Ch. marginalis* were recorded in the area between the forelimbs of one of the carcasses on Day 4 specifically in the early summer seasonal trial (Figure B17). During the mid-, and late summer trials, the eggs, first and/or second instar larvae of *Ch. megacephala* were recorded in the oral cavities and area around the forelimbs of the carcasses between Days 5 and 8 (Figures B19, B21 & B22). Freshly laid egg masses of *H. fernandica* were recorded in the area between the forelimbs of one of the carcasses on Day 3 during the late summer trials (Figure B22).

Across the early, mid-, and late summer trials, members of the family Sarcophagidae arrived on the pig carcasses between Days 1 and 2 and were generally present until the end of the experimental trials (Figures B17 – B22). The first and second instar larvae of this family were recorded in the oral cavities, and areas around the limbs and abdomen of the carcasses between Days 1 and 5 across the three summer seasonal trials (Figures B17 – B22). Members of the family Muscidae, specifically *Musca* sp., arrived on the carcasses between Days 1 and 3 across the three summer seasonal trials, whilst maintaining a constant presence on the carcasses until the termination of the trials (Figures B17 – B22). *Hydrotaea* sp. on the other hand, arrived between Days 5 and 8 mostly when the carcasses were in late phase of early decomposition and advanced decomposition stages across the three summer seasonal trials (Figures B17 – B22). As with *Musca* sp., *Hydrotaea* sp. persisted on the carcasses until the skeletonization stage across the three summer trials (Figures B17 – B22).

Members of the family Piophilidae and Sepsidae arrived on the carcasses mostly during the advanced decomposition stages (between Days 5 and 9) and were present on the carcasses until the skeletonization stage across the three summer trials (Figures B17 – B22). Members of other Dipteran families such as Drosophilidae, Anthomyiidae, and Sciomyzidae arrived on the carcasses during the early and advanced decomposition stages across the three summer trials and were present in varying numbers until the skeletonization stage (Figures B17 – B22).

Coleoptera succession

Members of the families Dermestidae, Silphidae, Histeridae, Tenebrionidae, Staphylinidae and Cleridae arrived on the carcasses during the late phase of early decomposition and onset of advanced decomposition stages between Days 5 and 8 across the early, mid-, and late summer trials (Figures B17 – B22). Members of these Coleopteran families including those belonging to the families Scarabaeidae were generally present on the carcasses albeit in varying abundance, following the transition into the skeletonization stage (Figures B17 – B22).

Hymenoptera and other insect orders succession

Crematogaster cf. liengmei was observed to arrive on the pig carcasses as early as Days 1 and 2 during the mid-, and late summer trials (Figures B19 – B22). However, this ant species was not recorded on the pig carcasses during the early summer trial. In the mid-, and late summer trials, they were neither observed to exhibit any predatory/necrophagous behavior, nor were they able to persist on the carcasses until the skeletonization stage (Figures B19 – B22). *Pheidole* sp. on the other hand was only recorded on one of the carcasses on Day 1 during the late summer trial (Figure B22).

The colonization pattern of *A. manducator* on the pig carcasses was highly variable across the three summer trials. This parasitoid wasp was noted to arrive on selected days after the establishment of larval masses on the carcasses during the early, advanced decomposition and skeletonization stages (Figures B17, B19, B21 & B22). As with *A. manducator*, individuals of the orders Hemiptera and Blattodea were seen randomly on the carcasses but only during the early and mid-summer trials respectively (Figures B17 – B19).

3.3. Autumn Season

3.3.1. Weather data

Early autumn

In this trial, mean internal and external carcass temperatures ranged between 18.2°C to 26.5°C (overall mean = 21.8°C) and 17°C to 25.6°C (overall mean = 21.3°C) respectively (Figure 3.18A). Daily mean ambient temperature ranged between 18°C to 22.7°C (overall mean = 20°C) (Figure 3.18A). Daily mean relative humidity ranged between 66% to 73.9% (mean = 68.9%), while daily rainfall ranged between 0.0mm to 5.5mm (overall mean = 0.7mm) (Figure 3.19A).

Mid-autumn

In this trial, mean internal and external carcass temperatures ranged between 11.5°C to 21.6°C (overall mean = 15.2°C) and 10°C to 22.8°C (overall mean = 14.8°C) respectively (Figure 3.18B). Daily mean ambient temperature ranged between 13°C to 23.3°C (overall mean = 16°C) (Figure 3.18B). Daily mean relative humidity ranged between 30.2% to 95.6% (overall mean = 75%), while daily rainfall ranged between 0.0mm to 63.1mm (overall mean = 8.5mm) (Figure 3.19B).

Late autumn

In this trial, mean internal and external carcass temperatures ranged between 11.1°C to 19.3°C (overall mean = 14.3°C) and 11°C to 22.2°C (overall mean = 14.6°C) respectively (Figure 3.18C). Daily mean ambient temperature ranged between 13°C to 24.5°C (overall mean = 16.6°C) (Figure 3.18C). Daily mean relative humidity ranged between 28.1% to 95.6% (overall mean = 69%), while daily rainfall ranged between 0.0mm to 63.1mm (overall mean = 7.4mm) (Figure 3.19C).

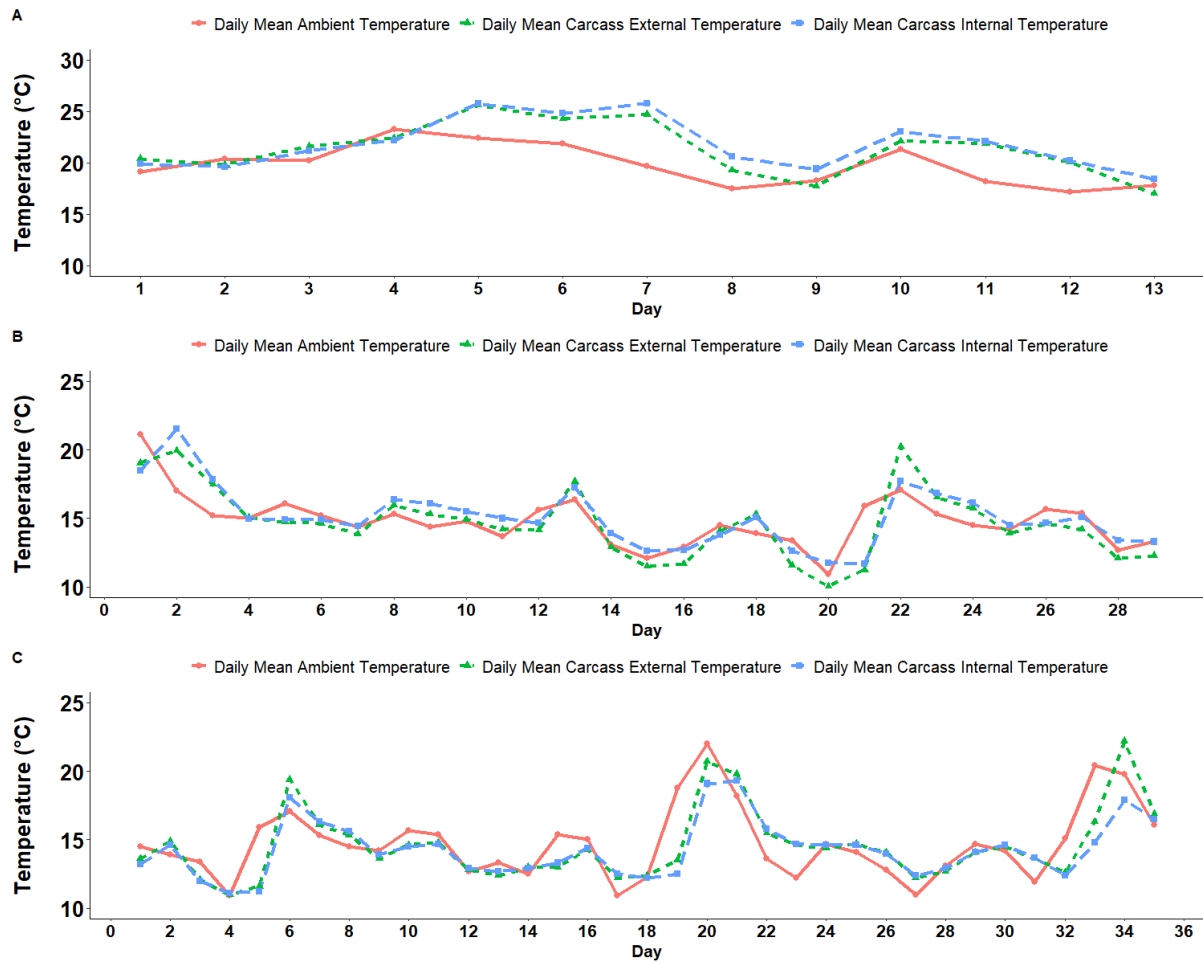


Figure 3.18: Daily mean ambient, external, and internal carcass temperatures in the early (A), mid- (B), and late autumn (C) neonate pig trials.

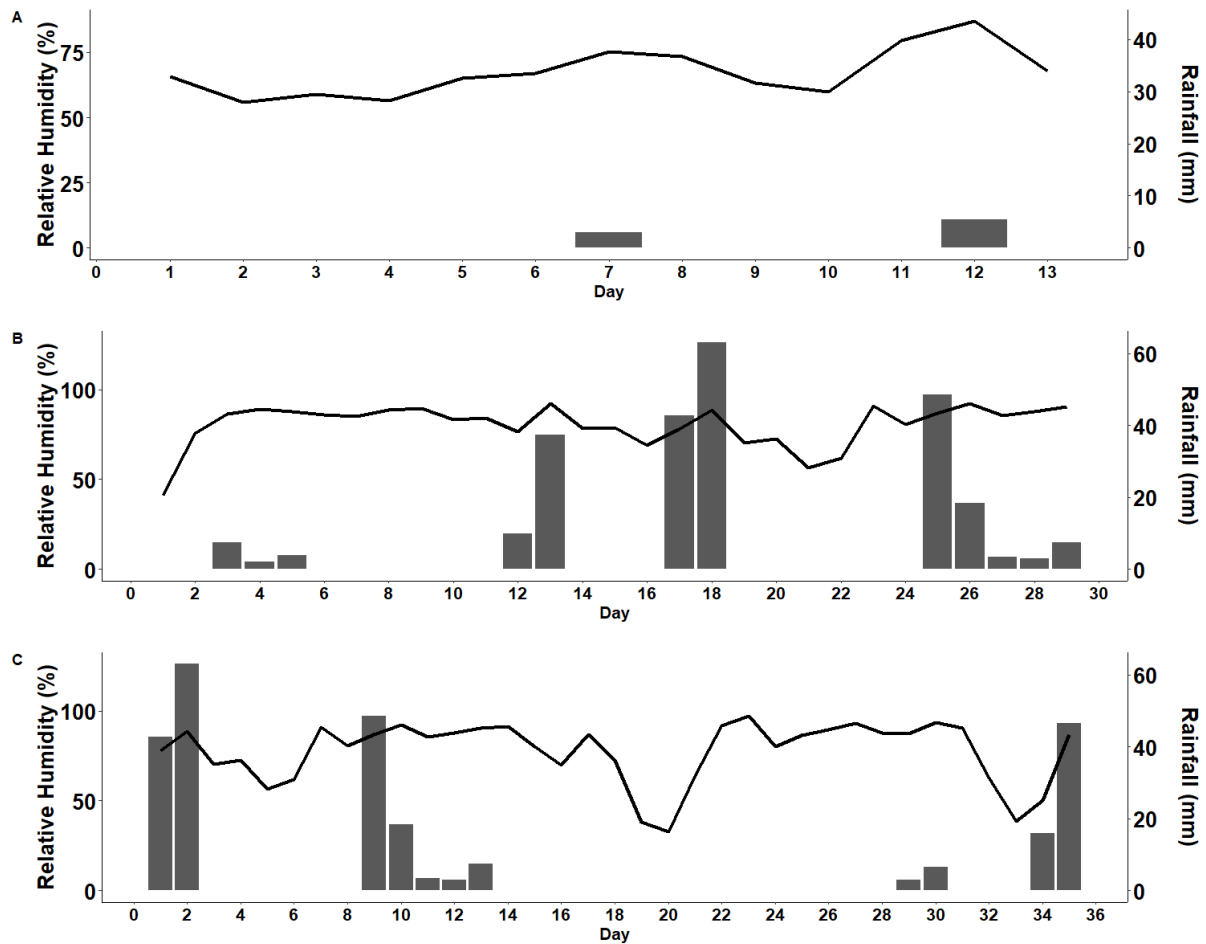


Figure 3.19: Daily mean ambient relative humidity (line) and rainfall (bars) during the early (A), mid- (B), and late autumn (C) neonate pig trials.

3.3.2. Decomposition data

Fresh Stage (TBS = 3): This stage commenced immediately after the thawing and deployment of the carcasses. Here, no changes in the coloration and appearance of the carcasses were observed (Figures 3.20A & 3.20E, B23A & B23E, B24A and B24E). In the early (ADD = 20.1) and mid-autumn (ADD = 18.8) trials, this stage lasted for only 1 Day. However, in the late autumn trial, the carcasses remained fresh for a period of 2 Days (ADD = 28.2).

Early Decomposition Stage (TBS: 4 – 16): This stage was marked by a slight change in the appearance of the carcasses' head, neck, and trunk. The head and neck became dark red in coloration. The abdominal region of the carcasses had a shiny appearance. Slight inflammation of the head, neck and abdominal regions of the carcasses was observed (Figures 3.20B & 3.20F, B23B & B23F, B24B and B24F). Furthermore, there was an increase in the presence and activities of insects in/on the carcasses especially around the trunks and limbs. Signs of skin

slippage and hair loss became obvious during the late phase of early decomposition. In addition, cadaveric fluids began to ooze from the oral cavities and abdominal regions of the carcasses into the underlying soil. In the early autumn trial, this stage began on Day 2 (ADD = 39.7) and ended on Day 6 (ADD = 133.7). In the mid-autumn trial, both carcasses transitioned into the early decomposition stage on Day 2 (ADD = 39.5) however, the decomposition stage came to an end in one of the carcasses on Day 10 (ADD = 162.9) and in the other on Day 14 (ADD = 222.7). In the late autumn trial, both carcasses transitioned into the early decomposition stage on Day 3 (ADD = 40.2), but the decomposition stage came to an end in one of the carcasses on Day 18 (ADD = 246.7) and in the other on Day 21 (ADD = 299.1).

Advanced Decomposition Stage (TBS: 17 – 24): This stage was marked by the shrinkage of the head and neck and deflation of the trunk. A significant amount of cadaveric fluids were seen oozing out from the abdominal region. Furthermore, the carcasses began to show some signs of extensive skin slippage, bone exposure and separations in the three body scoring regions (i.e., head and neck, trunk, and limbs) (Figures 3.20C & 3.20G, B23C & B23G, B24C and B24G). Blow fly and/or flesh fly larvae became concentrated in and underneath the abdominal regions of the carcasses. During the late phase of advanced decomposition, the carcasses began to show some signs of mummification accompanied by the migration of post-feeding larvae in different directions. This stage lasted for only three days in the early autumn trial beginning on Day 7 (ADD = 159.1) and ending on Day 9 (ADD = 198.1). In the mid-autumn trial, this stage was between Days 11 (ADD = 177.5) to Day 20 (ADD = 299.1) for one of the carcasses and Days 15 (ADD = 234.8) to 24 (ADD = 362.2) for the other carcass. In the late autumn trial, one of the carcasses transitioned into the advanced decomposition stage on Day 19 (ADD = 259.6) and the other on Day 22 (ADD = 314.8). The stage came to an end on Day 27 (ADD = 385) and 28 (ADD = 397.8) respectively for the two carcasses.

Skeletonization Stage (TBS $\geq$ 25): This stage was characterized by the mummification of the carcasses skin, significant loss of carcass abdominal contents, presence of bones, and grease substances (Figures 3.20D & 3.20H, B23D & B23H, B24D and B24H). During the early autumn trial, the carcasses transitioned into the skeletonization stage on Day 10 (ADD = 220.9). In the mid-autumn trial, this stage commenced on Day 21 (ADD = 310.6) in one of the carcasses and on Day 25 (ADD = 376.4) in the other carcass. In the late autumn trial, one of the carcasses transitioned into the skeletonization stage on Day 28 (ADD = 397.8) and the other on Day 29 (ADD = 411.9).



Figure 3.20: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the early autumn neonate pig trial.

3.3.3. Abundance and diversity of identified insect taxa

Early autumn

A total of 1,916 adult insect individuals belonging to four orders (Diptera, Hymenoptera, Coleoptera, and Orthoptera) were recorded. Amongst the insect orders recorded, the order Diptera (72.8%) was the most abundant followed by the orders Coleoptera (17.8%), Hymenoptera (9.2%), and Orthoptera (0.2%) (Figure 3.21A). Within the order Diptera, a total of 1,395 individuals were recorded of which members of the family Muscidae (38.7%) were the most abundant followed by the families Calliphoridae (31.5%), Sarcophagidae (12.4%), Piophilidae (12%), Sepsidae (4.2%) and fewer individuals (<1%) of the families Drosophilidae, Anthomyiidae, Heleomyzidae, Sciomyzidae and Scathophagidae (Figure 3.21B). Within the family Calliphoridae, a total of 439 Calliphorid flies were recorded of which *Ch. albiceps* (59.7%) was the most abundant followed by *Ch. megacephala* (28.7%), *L. cuprina* (4.1%), *H. fernandica* (2.7%), *L. sericata* (2.5%), and fewer individuals (<2%) of *Ch. marginalis*, and *Ch. chloropyga* (Figure 3.21C). Amongst the order Coleoptera, a total of 341 individuals were recorded of which the members of the family Silphidae (41.9%) were the most abundant, followed closely by the Histeridae (34%), Dermestidae (19.1%), and Staphylinidae (5%) (Figure 3.21D).

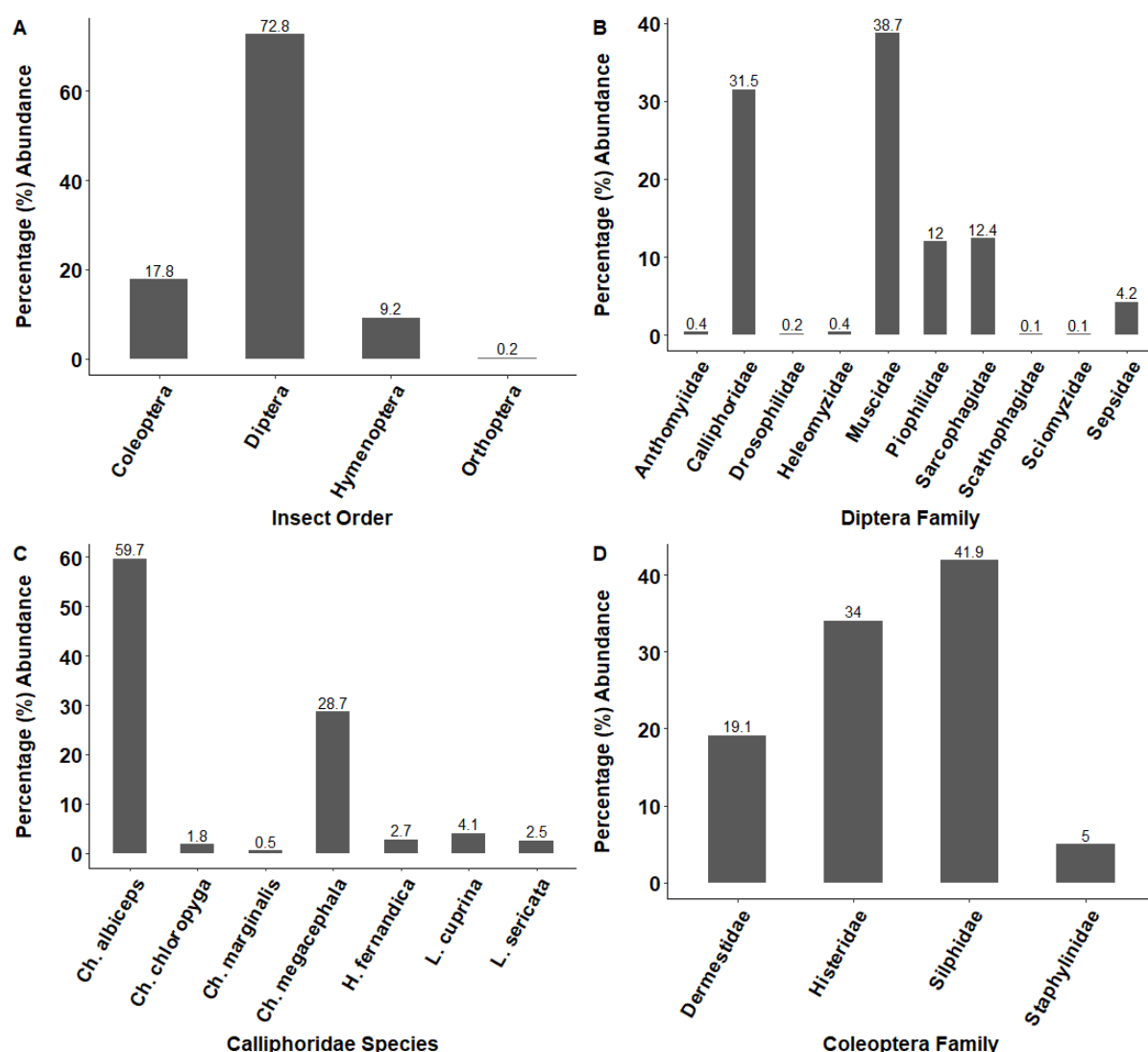


Figure 3.21: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the early autumn neonate pig trial.

Mid-autumn

A total of 3,136 adult insect individuals belonging to five orders (Diptera, Hymenoptera, Coleoptera, Mantodea and Blattodea) were recorded. Amongst the insect orders recorded, the order Diptera (75.2%) was the most abundant followed by the orders Hymenoptera (16.6%), Coleoptera (8%), Mantodea (0.1%) and Blattodea (0.1%) (Figure 3.22A). Within the order Diptera, a total of 2,357 individuals were recorded of which members of the family Muscidae (56.3%) were the most abundant followed by the families Calliphoridae (18.2%), Piophilidae (7.7%), Sarcophagidae (7.4%), Sepsidae (4.1%), Heleomyzidae (3.7%) and fewer individuals (<2%) of the families Drosophilidae, Anthomyiidae, Sciomyzidae Tephritidae and Scathophagidae (Figure 3.22B). Within the family Calliphoridae, a total of 424 Calliphorid

flies were recorded of which *Ch. albiceps* (45.8%) was the most abundant followed by *Ch. megacephala* (18.9%), *Ch. chloropyga* (12%), *H. fernandica* (9.4%), *Ca. croceipalpis* (7.5%), *Ch. marginalis* (5.4%), fewer individuals (<1%) of *L. cuprina* and *L. sericata* (Figure 3.22C). Amongst the order Coleoptera, a total of 252 individuals of which the members of the family Silphidae (38.5%) were the most abundant, followed closely by the Histeridae (36.5%), Staphylinidae (18.3%), Dermestidae (6.3%), and Scarabaeidae (0.4%) (Figure 3.22D).

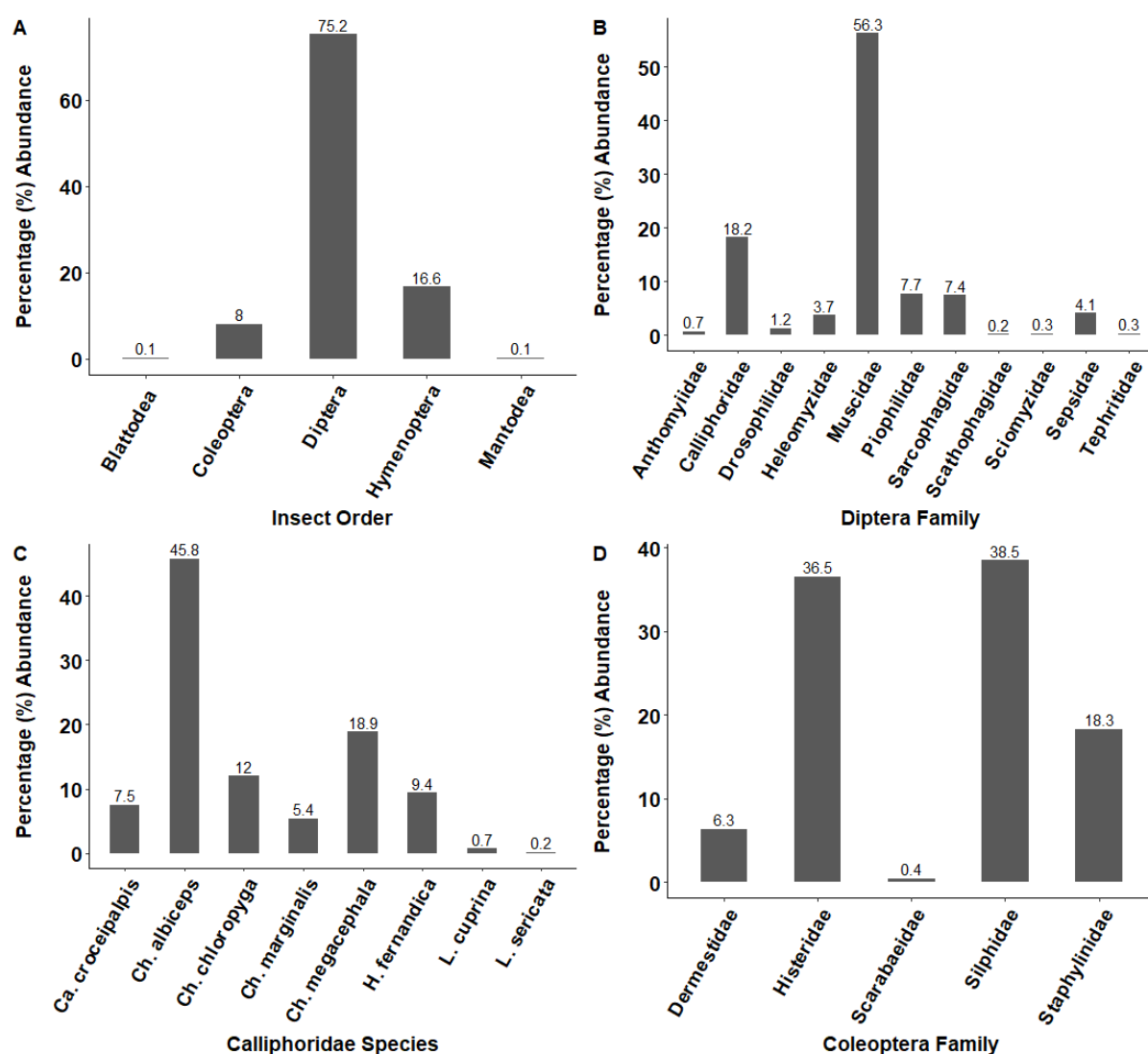


Figure 3.22: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the mid-autumn neonate pig trial.

Late autumn

A total of 2,986 adult insect individuals belonging to six orders (Diptera, Hymenoptera, Coleoptera, Hemiptera, Mantodea and Blattodea) were recorded. Amongst the insect orders recorded, the order Diptera (80.81%) was the most abundant followed by the order Hymenoptera (16.14%), Coleoptera (2.91%), and few individuals (<1%) of the orders Hemiptera, Mantodea and Blattodea (Figure 3.23A). Within the order Diptera, a total of 2,413 individuals were recorded of which members of the family Muscidae (53.83%) were the most abundant followed by the families Calliphoridae (22.09%), Sarcophagidae (8.25%), Piophilidae (8.12%), Sepsidae (3.56%), and fewer individuals (<2%) of the families Heleomyzidae, Ulidiidae, Drosophilidae, Anthomyiidae, Phoridae, Tephritidae and Scathophagidae (Figure 3.23B). Within the family Calliphoridae, a total of 533 Calliphorid flies were recorded of which *Ch. albiceps* (57.8%) was the most abundant followed by *Ch. megacephala* (19.3%), *Ca. croceipalpis* (6.6%), *H. fernandica* (6.2%), *Ch. chloropyga* (6%), *Ch. marginalis* (2.6%), fewer individuals (<2%) of *L. cuprina* and *L. sericata* (Figure 3.23C). Amongst the order Coleoptera, a total of 87 individuals of which the representatives of the family Staphylinidae (32.2%) were the most abundant, followed closely by the Silphidae (31%), Dermestidae (16.1%), Histeridae (10.3%), Carabidae (9.2%) and Scarabaeidae (1.1%) (Figure 3.23D).

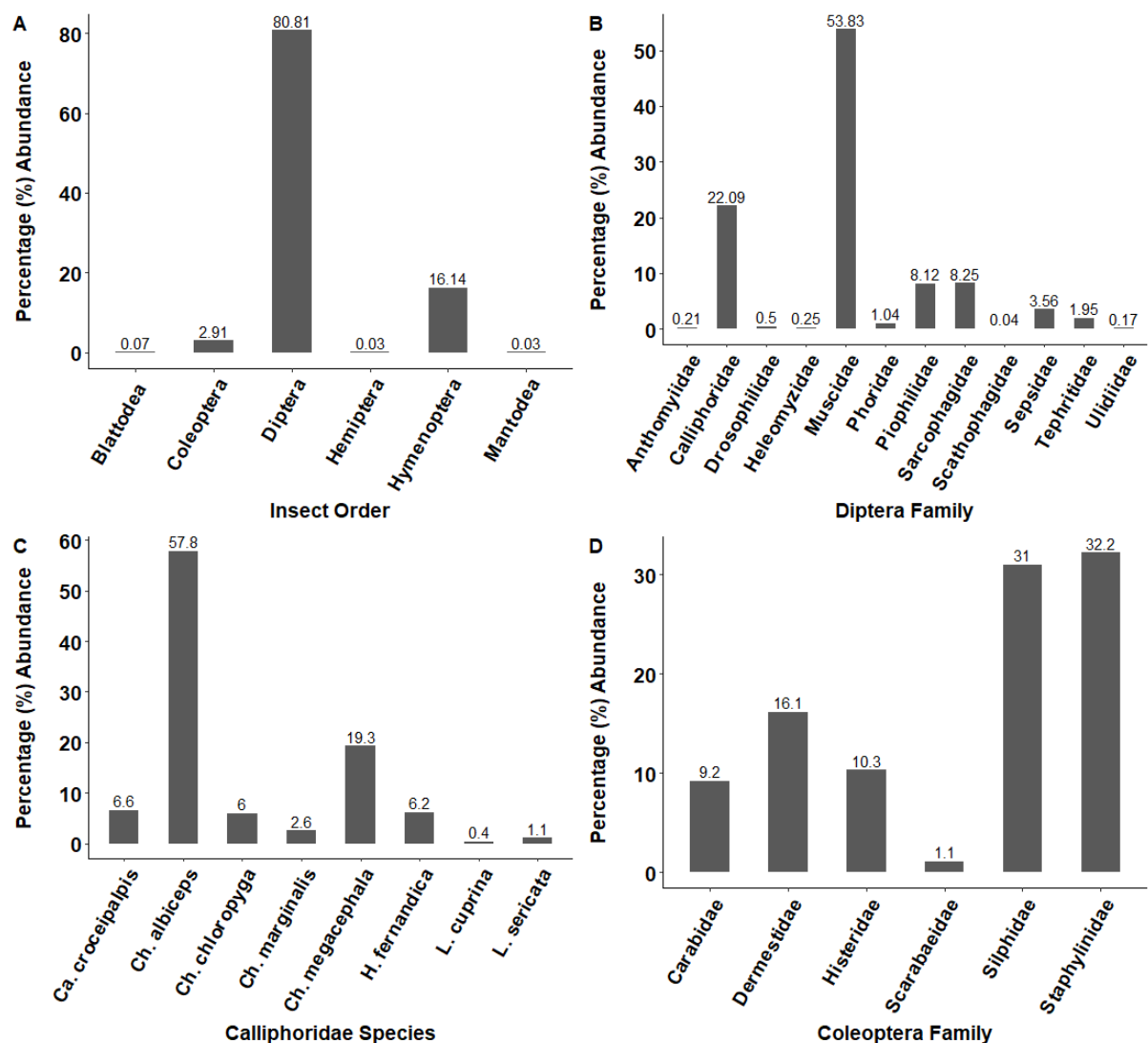


Figure 3.23: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the late autumn neonate pig trial.

3.3.4. Insect diversity and abundance across various decomposition stages

In all the three trials conducted in this autumn study, the number of insect species in relation to the decomposition stages significantly differed in the early (Likelihood Ratio $\chi^2 = 27.8$; DF = 3; $p < 0.0001$), mid- (Likelihood Ratio $\chi^2 = 35.4$; DF = 3; $p < 0.0001$), and late (Likelihood Ratio $\chi^2 = 67$; DF = 3; $p < 0.0001$) autumn trials. In the three autumn trials, the advanced decomposition and skeletonization stages attracted significantly higher number of insect species ($p < 0.05$) when compared to other stages of decomposition (Figures B25A, B26A, B27A). Similarly, there was a significant difference in the total number of insect individuals with respect to the decomposition stages in the early (Likelihood Ratio $\chi^2 = 534.7$; DF = 3; $p < 0.0001$), mid- (Likelihood Ratio $\chi^2 = 829.5$; DF = 3; $p < 0.0001$), and late (Likelihood Ratio

$\chi^2 = 760.8$; DF = 3; $p < 0.0001$) autumn trials. As it is common with the number of insect species, the advanced decomposition and skeletonization stage attracted the highest number of insect individuals ($p < 0.05$) in the three autumn trials (Figures B25B, B26B, B27B).

Insect diversity and abundance across various decomposition stages (combined data)

Combining the results obtained from the three trials within the autumn season, the number of insect species differed significantly in relation to the decomposition stages (Likelihood Ratio $\chi^2 = 132.7$; DF = 3; $p < 0.0001$). After a post-hoc Tukey test, the advanced decomposition and skeletonization stages were found to attract significantly higher number of insect species ($p < 0.05$) when compared to the fresh and early decomposition stages (Figure 3.24). Pertaining to the total number of insect individuals across the three monthly trials within the season, there was a statistically significant difference in relation to the decomposition stages (Likelihood Ratio $\chi^2 = 534.7$; DF = 3; $p < 0.0001$). Across the four decomposition stages in all the three trials, the advanced decomposition and skeletonization stages attracted the highest number of insect individuals ($p < 0.05$) (Figure 3.25A). The total number of insect individuals differed significantly across the three sampling trials (Likelihood Ratio $\chi^2 = 49.2$; DF = 2; $p < 0.0001$). After a post-hoc Tukey test, the total number of insect individuals in the early autumn trial was significantly higher ($p < 0.05$) than that of the mid- and late autumn trials (Figure 3.25B). There was a statistically significant interaction between the stages of decomposition and sampling trials (Likelihood Ratio $\chi^2 = 248.8$; DF = 6; $p < 0.0001$).

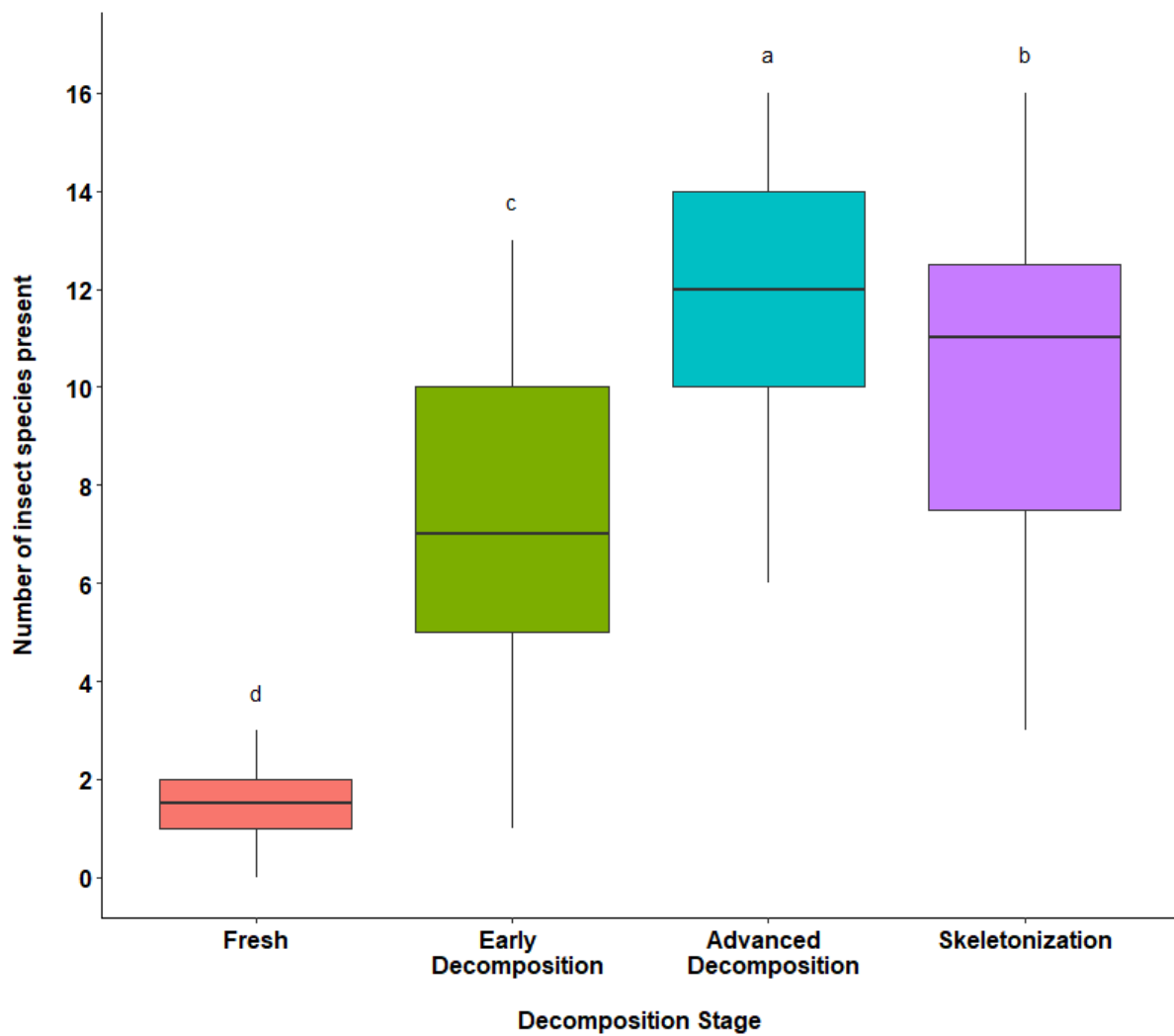


Figure 3.24: Box plot comparing the number of insect species present across the four decomposition stages in all the three neonate pig autumn seasonal trials. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

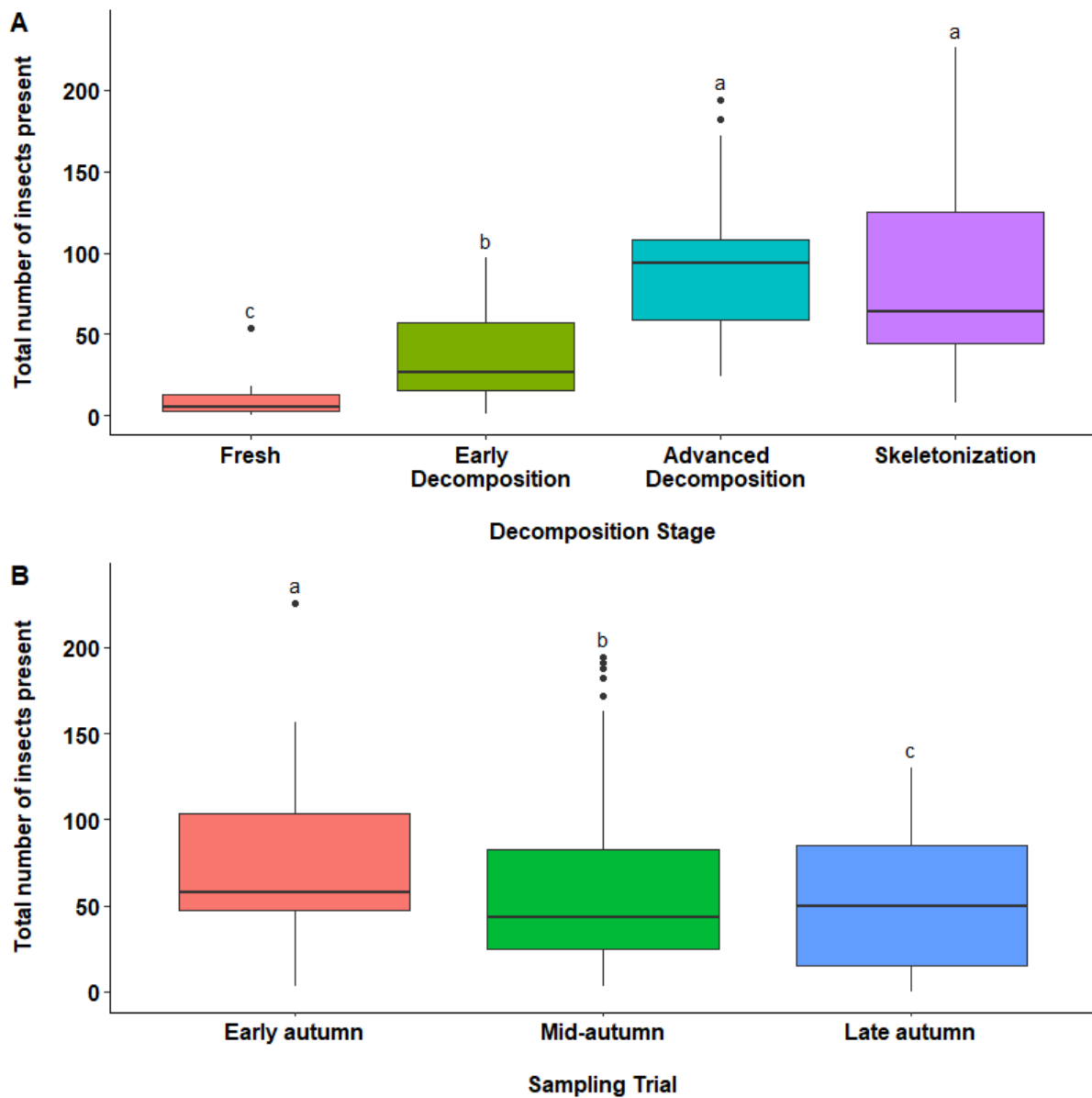


Figure 3.25: Box plots comparing the total number of insect individuals present across the four decomposition stages in all the three neonate pig autumn seasonal trials (A) and total number of insect individuals present across the three autumn trials (B). Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

3.3.5. Insect succession

Diptera succession

While the carcasses were still fresh (Day 1), no Calliphorid fly was observed to be present specifically in the early, and mid-autumn trials (Figures B28 – B31). However, in the late autumn trial, *Ca. croceipalpis* was observed to be the first Calliphorid fly to be present, arriving as early as Day 1 on one of the carcasses (Figure B33). Following the transition of the carcasses into the early decomposition stage (from Day 2) across the three autumn trials, several Calliphorid flies such as *Ch. megacephala*, *H. fernandica*, *Ch. albiceps*, *Ch. chloropyga*, *Ch. marginalis*, *L. sericata*, and *L. cuprina* (including *Ca. croceipalpis*) began to arrive on the carcasses (Figures B28 – B33). The abundance and time in which these Calliphorid flies spent on the carcasses varied across the early, mid-, and late autumn trials, with some species persisting until the skeletonization stage (Figures B28 – B33).

The eggs, and first instar larvae of *H. fernandica* were recorded in the areas around the forelimbs and abdomen of the carcasses between Days 2 and 6 across the mid-, and late autumn trials (Figures B30, B32 & B33). However, in the early autumn trials, the immature stages of *H. fernandica* were not recorded on the carcasses. The eggs, first and second instar larvae of *Ch. chloropyga* were recorded in the oral cavities and abdominal regions of the carcasses between Days 4 and 8, but only in the early and mid-autumn trials (Figures B28 – B30).

Across the early, mid-, and late autumn trials, members of the family Sarcophagidae were observed to arrive on the carcasses between Days 1 and 4 (Figures B28 – B33). Following their arrival, members of this Dipteran family persisted on the carcasses until the skeletonization stage (Figures B28 – B33). The first and second instar larvae of this family were recorded on the abdominal regions and in the oral cavities of the carcasses between Days 3 and 4 during the early and mid-autumn trials (Figures B28 & B31).

Musca sp. arrived on the carcasses between Day 3 and 7 across the early, mid-, and late autumn trials (Figures B28 – B33). In the three autumn trials, this Muscid fly persisted on both carcasses until the termination of the trials (Figures B28 – B33). *Hydrotaea* sp. on the other hand only arrived on the carcasses majorly during the late phase of early decomposition and advanced decomposition stages (between Days 8 and 25) and was present until the end of the trials (Figures B28 – B33). *Neomyia viridescens* was only present on one of the carcasses during the mid-autumn trial on Days 5 and 6 (Figure B31).

Across the three autumn trials, members of the family Piophilidae arrived on the carcasses during the late phase of early decomposition and early phase of advanced decomposition and further persisted until the termination of the trials (Figures B28 – B33). Across the early, mid-, and late autumn trials, *S. picta* was present on the carcasses on different days and their occurrence had no specified pattern (Figures B28 – B33). The colonization patterns and abundance of the members of other Dipteran families such as Drosophilidae, Ulidiidae, Anthomyiidae, Sciomyzidae, Sepsidae, Sciomyzidae, Scathophagidae, Phoridae, and Tephritidae on the carcasses were considerably variable across the three autumn trials (Figures B28 – B33).

Coleoptera succession

Across the three autumn trials, members of the families Histeridae, Silphidae, Staphylinidae, and Scarabaeidae arrived on the carcasses during the early decomposition stage (between Days 5 and 15) (Figures B28 – B33). Members of the family Carabidae were only noted on one of the carcasses during the late autumn trial (Figure B33). As the carcasses transitioned into the advanced decomposition and skeletonization stages, members of the family Dermestidae began to arrive on the carcasses across the early, mid-, and late autumn trials (Figures B28 – B33).

The abundance of these Coleopteran families on the carcasses varied, and some families were able to persist on the carcasses until the termination of the trials (Figures B28 – B33). Of all the beetles that were present on the carcasses, only the larvae of Silphid beetles were recorded. The Silphid beetle larvae were recorded on the carcasses in the mid-, and late autumn trials across the advanced decomposition and skeletonization stages (Figures B30 – B32).

Hymenoptera and other insect orders succession

In the early, mid-, and late autumn trials, *Crematogaster* cf. *liengmei* was observed to arrive on the carcasses between Days 1 and 2 (Figures B28 – B33). Following their arrival on the carcasses in the three autumn trials, this ant species maintained a constant presence on the carcasses until the skeletonization stage (Figures B28 – B33). In the mid-autumn trial, several *Crematogaster* cf. *liengmei* individuals were seen feeding voraciously on the flesh around limbs, ears, noses, and abdominal regions of the pig carcasses (Figures 3.26A – 3.26C). Their feeding activities resulted in the creation of several bite marks and lesions on the carcasses (Figures 3.26C & 3.26D), some of which were exploited by blow flies as oviposition sites (Figures 3.27A & 3.27B). Furthermore, some individuals of *Crematogaster* cf. *liengmei* were

recorded preying on the eggs of Calliphorid flies deposited in and around the lesions (Figures 3.27A & 3.27B).

Alysia manducator arrived on one of the carcasses during the early decomposition and advanced decomposition stages (between Days 8 – 11) across the three autumn trials, and as with *Crematogaster* cf. *liengmei*, they were able to maintain a constant presence on the carcasses until the skeletonization stage (Figures B28 – B33). Individuals of other less frequent insect families and orders such as Apidae, Mantodea, Blattodea, Hemiptera, Orthoptera were recorded on and around the carcasses across the three autumn trials (Figures B28 – B33).

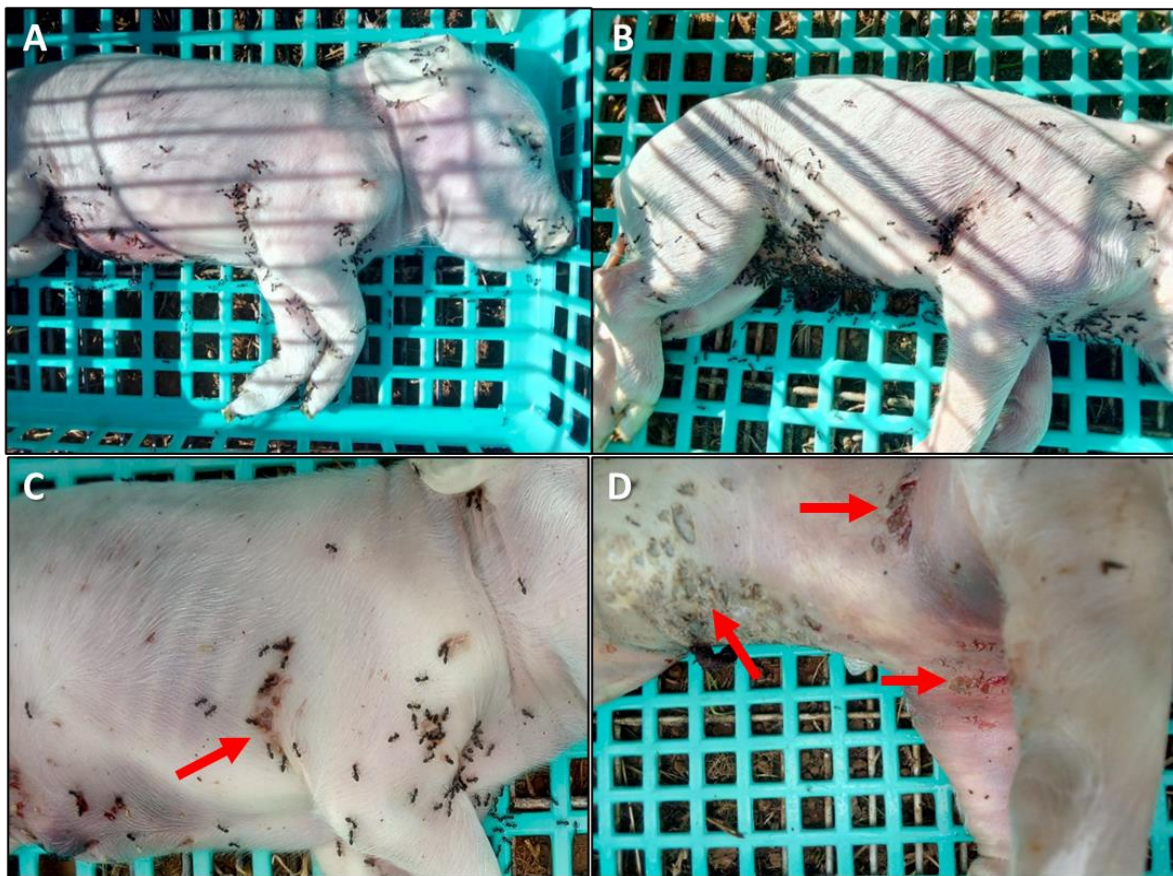


Figure 3.26: Several individuals of *Crematogaster* cf. *liengmei* aggregating in and around several regions of the carcasses on Day 2 during the mid-autumn trial (A and B). Moist and fleshy lesions (red arrows) inflicted on the carcasses due to the feeding activities of *Crematogaster* cf. *liengmei* (C and D) (Adapted from Adetimehin *et al.*, 2022).

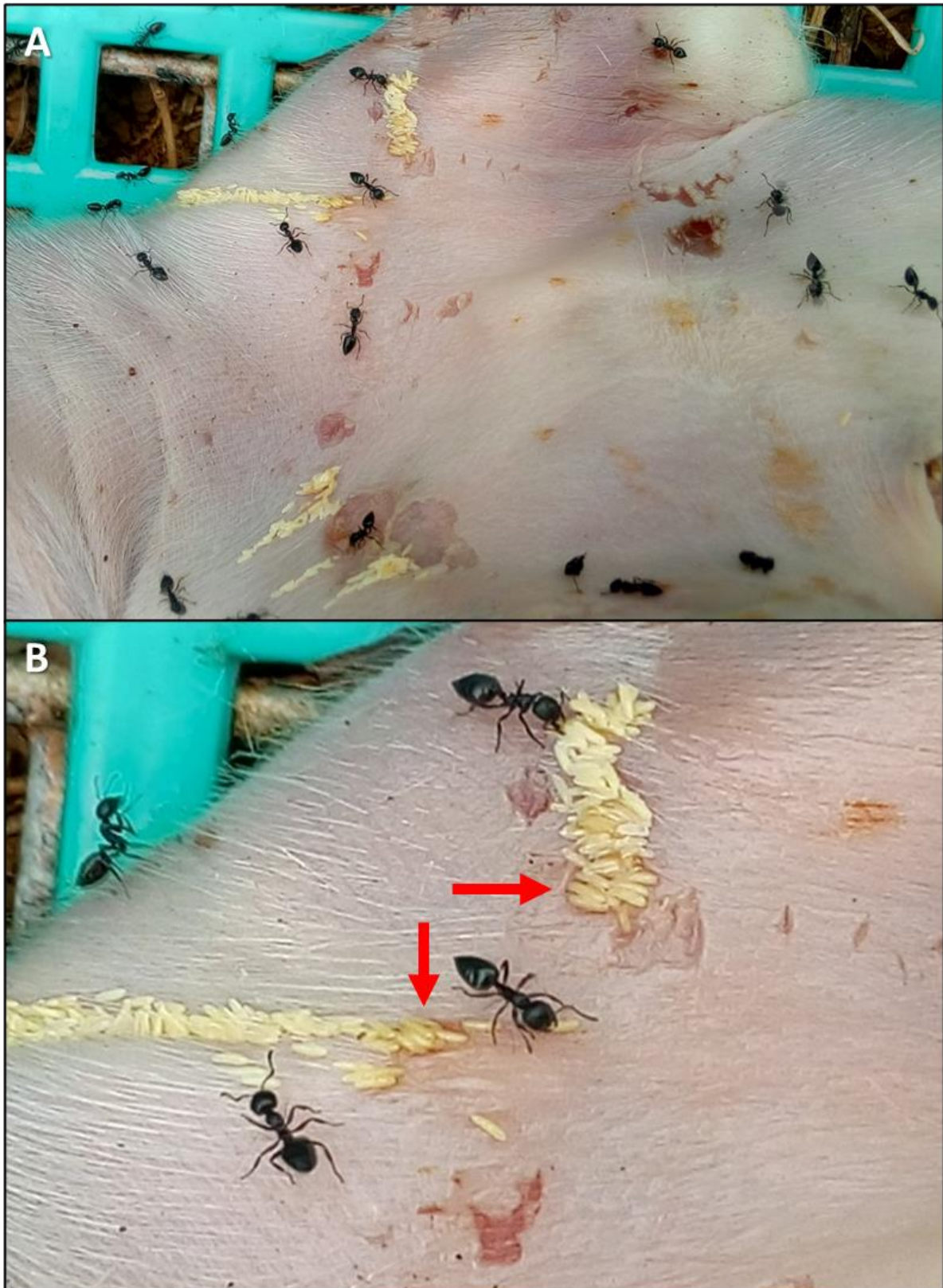


Figure 3.27: Distant and close-up views of *Crematogaster* cf. *liengmei* predating on the eggs (red arrows) of Calliphoridae flies deposited in and around the fleshy ant-inflicted lesions (A and B) (Adapted from Adetimehin *et al.*, 2022).

3.4. Winter Season

3.4.1. Weather data

Early winter

In this trial, the mean external carcass temperature ranged between 7.6°C to 17.1°C (overall mean = 12°C) (Figure 3.28A). Daily mean ambient temperature ranged between 9.2°C to 20.1°C (overall mean = 13.3°C) (Figure 3.28A). Daily mean relative humidity ranged between 29.5% to 92.6% (overall mean = 75%), while daily rainfall ranged between 0.0mm to 73.5mm (overall mean = 10.2mm) (Figure 3.29A).

Mid-winter

In this trial, mean internal and external carcass temperatures ranged between 7.3°C to 19°C (overall mean = 12.4°C) and 7.4°C to 19.5°C (overall mean = 13.1°C) respectively (Figure 3.28B). Daily mean ambient temperature ranged between 9.2°C to 20.1°C (overall mean = 14.5°C) (Figure 3.28B). Daily mean relative humidity ranged between 29.5% to 92.3% (overall mean = 66.1%), while daily rainfall ranged between 0.0mm to 45.2mm (overall mean = 5mm) (Figure 3.29B).

Late winter

In this trial, mean internal and external carcass temperatures ranged between 7.2°C to 19°C (overall mean = 13.2°C) and 7.3°C to 18°C (overall mean = 13.2°C) respectively (Figure 3.28C). Daily mean ambient temperature ranged between 8.2°C to 19.9°C (overall mean = 13.7°C) (Figure 3.28C). Daily mean relative humidity ranged between 55% to 95.8% (overall mean = 74.7%), while daily rainfall ranged between 0mm to 65mm (overall mean = 7mm) (Figure 3.29C).



Figure 3.28: Daily mean ambient, external, and internal carcass temperatures in the early (A), mid- (B), and late winter (C) neonate pig trials.

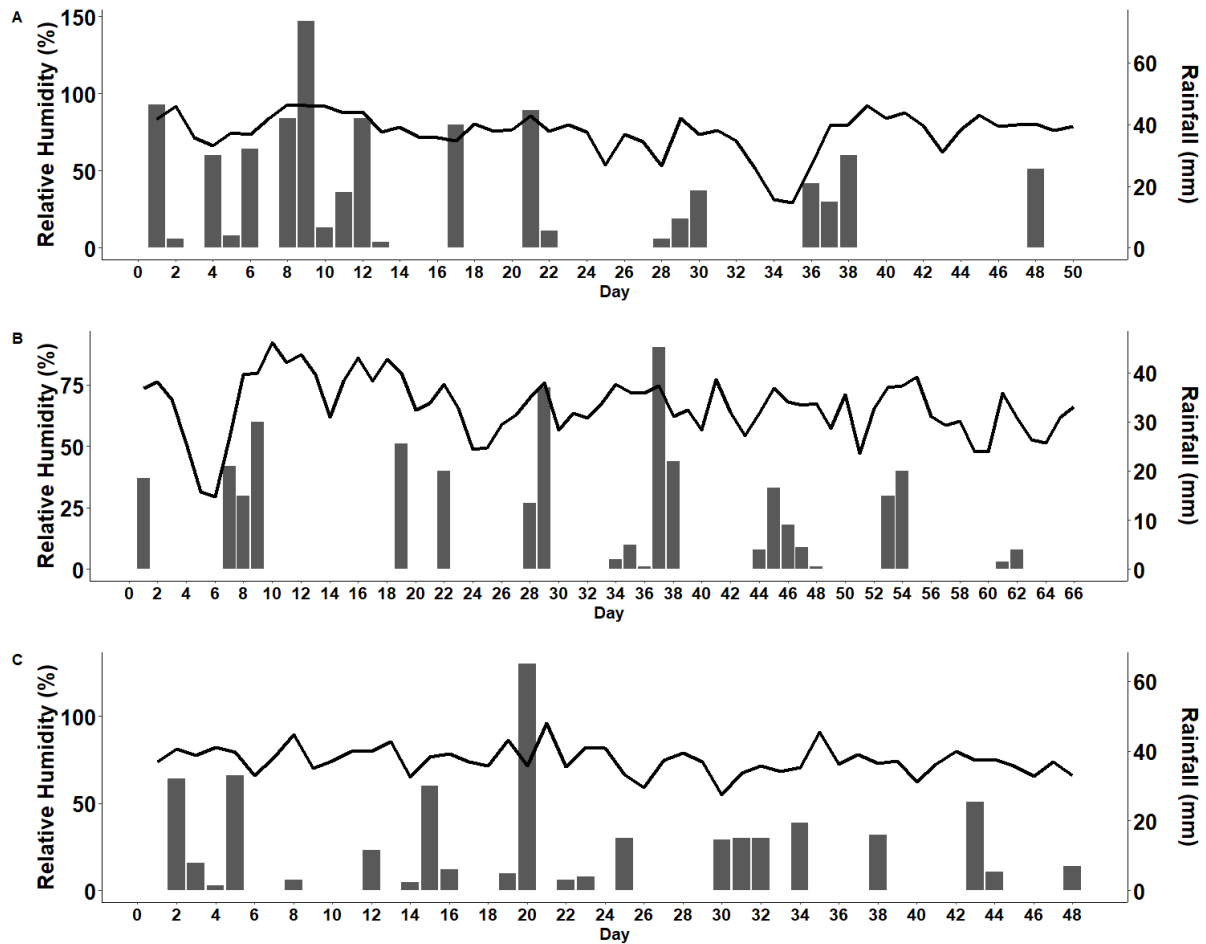


Figure 3.29: Daily mean ambient relative humidity (line) and rainfall (bars) during the early (A), mid- (B), and late winter (C) neonate pig trials.

3.4.2. Decomposition data

Fresh Stage (TBS = 3): This stage began the moment after the thawing and placement of the carcasses in the study sites. No observable changes in the color and appearance of the carcasses were noted (Figures 3.30A & 3.30E, B34A & B34E, B35A & B35E). In the early winter trial, this stage lasted for 1 Day (ADD = 16.6), while in the mid- (ADD = 17) and late (ADD = 26.7) winter trials, the carcasses were in the fresh stage for 2 days.

Early Decomposition Stage (TBS: 4 – 16): This stage was characterized by a small change in the appearance of the 3 scoring regions (i.e., head, neck, and trunk) of the carcasses. The head and neck began to appear slightly dark red to purple in coloration. The head, neck and abdominal regions of the carcasses further had a glossy appearance and were slightly bloated (Figures 3.30B & 3.30F, B34B & B34F, B35B & B35F). Adult and immature insects (i.e., larvae) became active in and around the carcasses. During the late phase of early

decomposition, loss of hair, tearing of the skin, and seepage of cadaveric fluids from the carcasses became conspicuous. In the early winter trial, both carcasses transitioned into the early decomposition stage on Day 2 (ADD = 30.4). This stage came to an end in both carcasses on Day 21 (ADD = 257.2). In the mid-winter trial, both carcasses transitioned into the early decomposition stage on Day 3 (ADD = 24.7), and this stage came to an end in both carcasses on Day 35 (ADD = 429.2). In the late winter trial, both carcasses transitioned into the early decomposition stage on Day 3 (ADD = 35.9), but the decomposition stage came to an end in one of the carcasses on Day 17 (ADD = 206.3) and in the other carcass on Day 18 (ADD = 215.1).

Advanced Decomposition Stage (TBS: 17 – 24): This stage was characterized by the caving in of the flesh of eyes, head, neck, and trunk. In addition, the carcasses began to show some signs of extensive skin slippage, bone exposure and disarticulations in the skeletal parts of head and neck, and limbs (Figures 3.30C & 3.30G, B34C & B34G, B35C & B35G). Fly larval feeding activities became concentrated between the forelimbs and underneath the abdominal regions of the carcasses. This led to an increase in the release of decomposition fluids around and underneath the carcass. The skin of one of the carcasses in the late winter trial had a dry and leathery appearance. This resulted in the skin forming a shell-like structure over the upper and lower abdominal regions and limbs of the carcass. In the early winter trial, both carcasses transitioned into the advanced decomposition stage on Day 22 (ADD = 265.1). However, this stage came to an end on one of the carcasses on Day 41 (ADD = 486.1) and in the other on Day 43 (ADD = 513.1). In the mid-winter trial, this stage was between Days 36 (ADD = 442.4) to Day 56 (ADD = 694.5) for both carcasses. In the late winter trial, one of the carcasses transitioned into the advanced decomposition stage on Day 18 (ADD = 215.1) and the other on Day 19 (ADD = 224.4). The stage came to an end on Day 40 (ADD = 526.8) and Day 34 (ADD = 447.3) respectively for the two carcasses.

Skeletonization Stage (TBS $\geq$ 25): This stage was characterized by the dehydration of the carcasses skin, coupled with significant loss of carcass abdominal tissues and hooves of the limbs. In addition, a mixture of dry bones and greasy substances were observed around and underneath the abdominal region (Figures 3.30D & 3.30H, B34D & B34H, B35D & B35H). In the late winter trial, one of the carcasses had its skin forming a dry shell-like leathery structure over the exposed region of the abdomen and limbs. However, bone exposure was apparent around the head and the region of the abdomen in contact with the soil. During the early winter trial, one of the carcasses transitioned into the skeletonization stage on Day 42

(ADD = 500), while the other carcass transitioned on Day 44 (ADD = 527.9). In the mid-winter trial, both carcasses transitioned into the skeletonization stage on Day 57 (ADD = 706.6). In the late winter trial, one of the carcasses transitioned into the skeletonization stage on Day 35 (ADD = 459.4) and the other on Day 41 (ADD = 542.6).



Figure 3.30: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the early winter neonate pig trial.

3.4.3. Abundance and diversity of identified insect taxa

Early winter

A total of 1,624 adult insect individuals belonging to six orders (Diptera, Hymenoptera, Coleoptera, Blattodea, Mantodea, and Orthoptera) were recorded. Amongst the insect orders recorded, the order Diptera (47%) was the most abundant followed by the orders Hymenoptera (33%), Coleoptera (17.4%), Blattodea (2.3%), Orthoptera and Mantodea (both <1%) (Figure 3.31A). Within the order Diptera, a total of 764 individuals were recorded of which members of the family Piophilidae (27.6%) were the most abundant followed by the families Phoridae (15.3%), Drosophilidae (13.7%), Muscidae (13%), Calliphoridae (9.3%), Sepsidae (8.2%) and fewer individuals (<5%) of the families Sarcophagidae, Anthomyiidae, Heleomyzidae, Tephritidae, and Curtonotidae (Figure 3.31B). Within the family Calliphoridae, a total of 71 Calliphorid flies were recorded of which *Ca. croceipalpis* (47.9%) was the most abundant followed by *Ch. albiceps* (22.5%), *Ch. chloropyga* (15.5%), *Ch. megacephala* (11.3%), fewer individuals (<2%) of *H. fernandica*, and *Ca. vicina* (Figure 3.31C). Amongst the order Coleoptera, a total of 282 individuals of which the members of the family Silphidae (88.7%) were the most abundant, followed by the families Staphylinidae (2.8%), Tenebrionidae (2.1%), Scarabaeidae (2.1%) and fewer individuals (<2%) of the families Dermestidae, Histeridae, Carabidae, and Trogidae (Figure 3.31D).

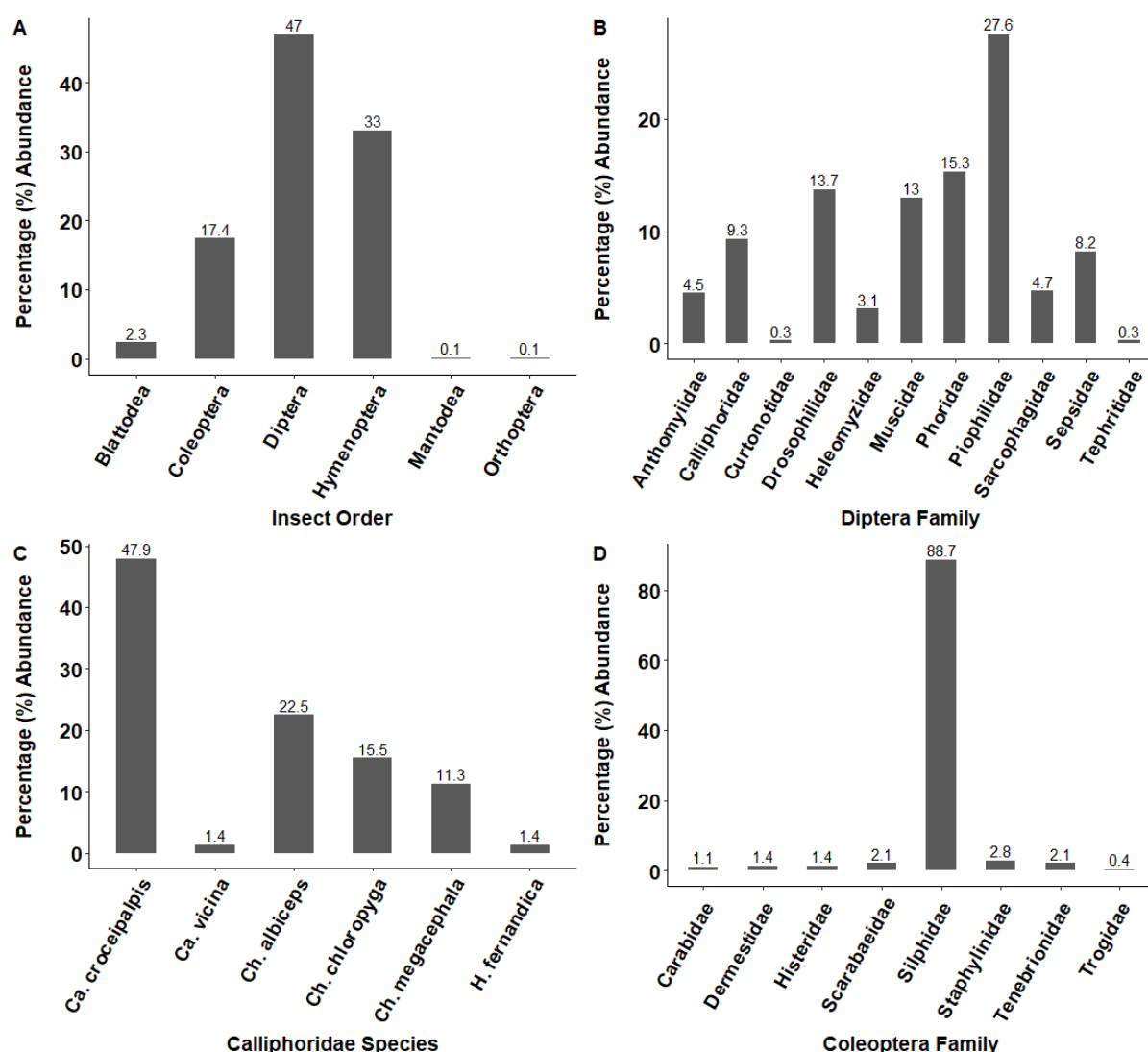


Figure 3.31: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the early winter neonate pig trial.

Mid-winter

A total of 4,001 adult insect individuals belonging to seven orders (Diptera, Hymenoptera, Coleoptera, Blattodea, Hemiptera, Mantodea, and Orthoptera) were recorded. Amongst the insect orders recorded, the order Diptera (56.04%) was the most abundant followed by the orders Hymenoptera (24.49%), Coleoptera (15.02%), Blattodea (4.22%), and fewer individuals (<1%) of the orders Hemiptera, Orthoptera and Mantodea (Figure 3.32A). Within the order Diptera, a total of 2242 individuals were recorded of which members of the family Drosophilidae (30.82%) were the most abundant followed closely by the families Calliphoridae (30.69%), Piophilidae (21.99%), Muscidae (6.29%), Sepsidae (4.68%) and fewer individuals (<2%) of the families Sarcophagidae, Anthomyiidae, Phoridae, Heleomyzidae, Tephritidae,

Curtonotidae, Scathophagidae, and Ulidiidae (Figure 3.32B). Within the family Calliphoridae, a total of 688 Calliphorid flies were recorded of which *Ch. chloropyga* (84.7%) was the most abundant followed by *Ch. albiceps* (10.8%), *Ca. croceipalpis* (3.2%), fewer individuals (<1%) of *Ch. megacephala*, *L. sericata*, and *Ca. vicina* (Figure 3.32C). Amongst the order Coleoptera, a total of 601 individuals of which the members of the family Silphidae (53.1%) were the most abundant, followed by the families Dermestidae (26.8%), Histeridae (12.1%), Staphylinidae (4.3%), Carabidae (2.8%) and fewer individuals (<1%) of the families Scarabaeidae, and Tenebrionidae (Figure 3.32D).

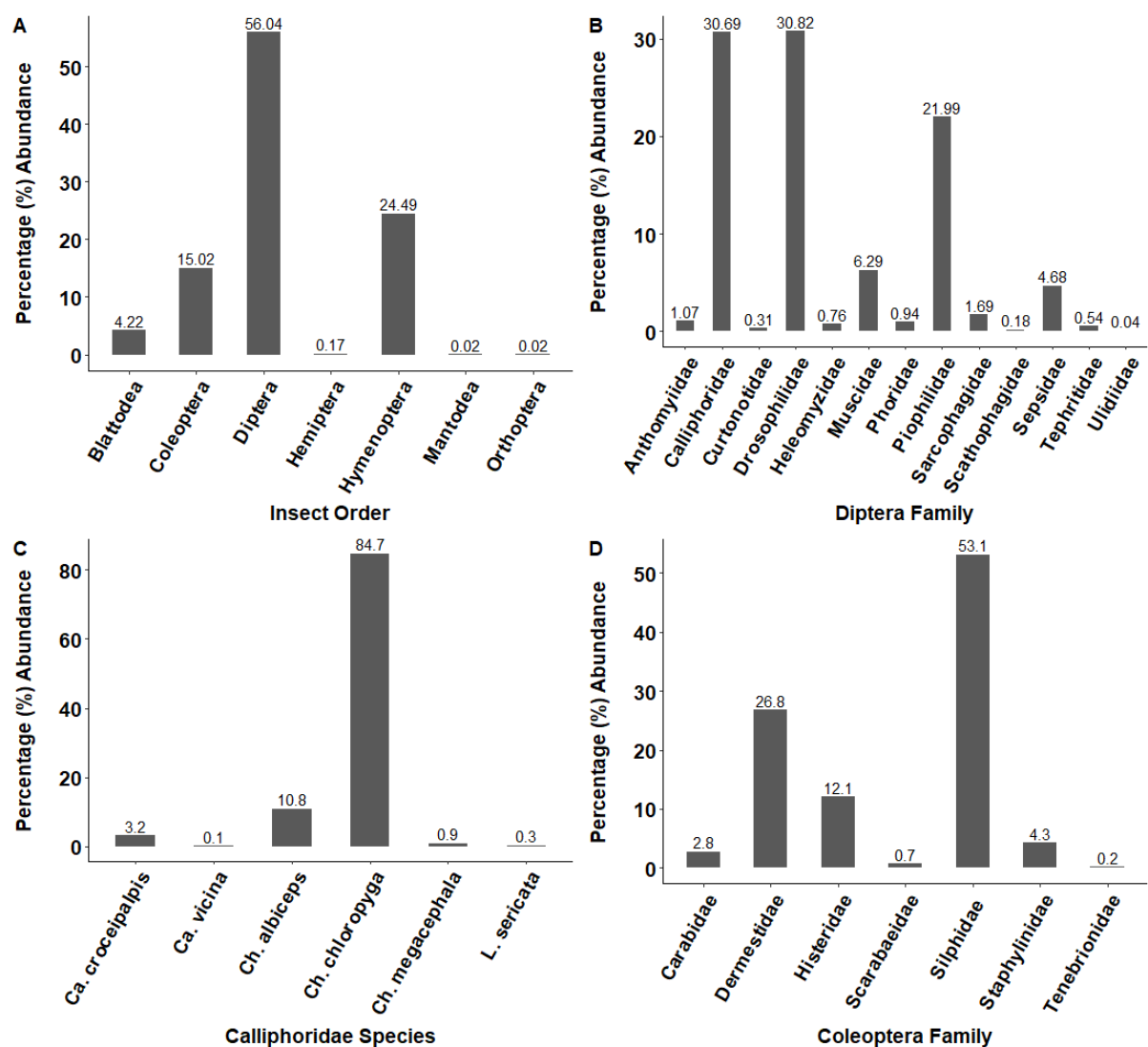


Figure 3.32: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the mid-winter neonate pig trial.

Late winter

A total of 6,130 adult insect individuals belonging to six orders (Diptera, Hymenoptera, Coleoptera, Blattodea, Hemiptera, and Orthoptera) were recorded. Amongst the insect orders recorded, the order Diptera (71.5%) was the most abundant followed by the orders Hymenoptera (23.5%), Coleoptera (4.6%), and fewer individuals (<1%) of the orders Blattodea, Hemiptera, and Orthoptera (Figure 3.33A). Within the order Diptera, a total of 4,385 individuals were recorded of which members of the family Calliphoridae (40.55%) were the most abundant followed by the families Muscidae (22.99%), Drosophilidae (12.45%), Heleomyzidae (8.51%), Piophilidae (8.42%), Phoridae (5.56%) and fewer individuals (<2%) of the families Sarcophagidae, Anthomyiidae, and Tephritidae (Figure 3.33B). Within the family Calliphoridae, a total of 1,756 Calliphorid flies were recorded of which *Ch. chloropyga* (73.2%) was the most abundant followed by *Ca. croceipalpis* (13.8%), *Ch. albiceps* (6.7%), *Ch. megacephala* (5.4%) and fewer individuals (<1%) of *Ch. marginalis*, *L. sericata*, *L. cuprina*, and *Ca. vicina* (Figure 3.33C). Amongst the order Coleoptera, a total of 281 individuals of which the members of the family Silphidae (36.3%) were the most abundant, followed by the families Histeridae (31.3%), Dermestidae (20.6%), Trogidae (6%), Scarabaeidae (5%) and fewer individuals (<1%) of the families Staphylinidae, and Coccinellidae (Figure 3.33D).

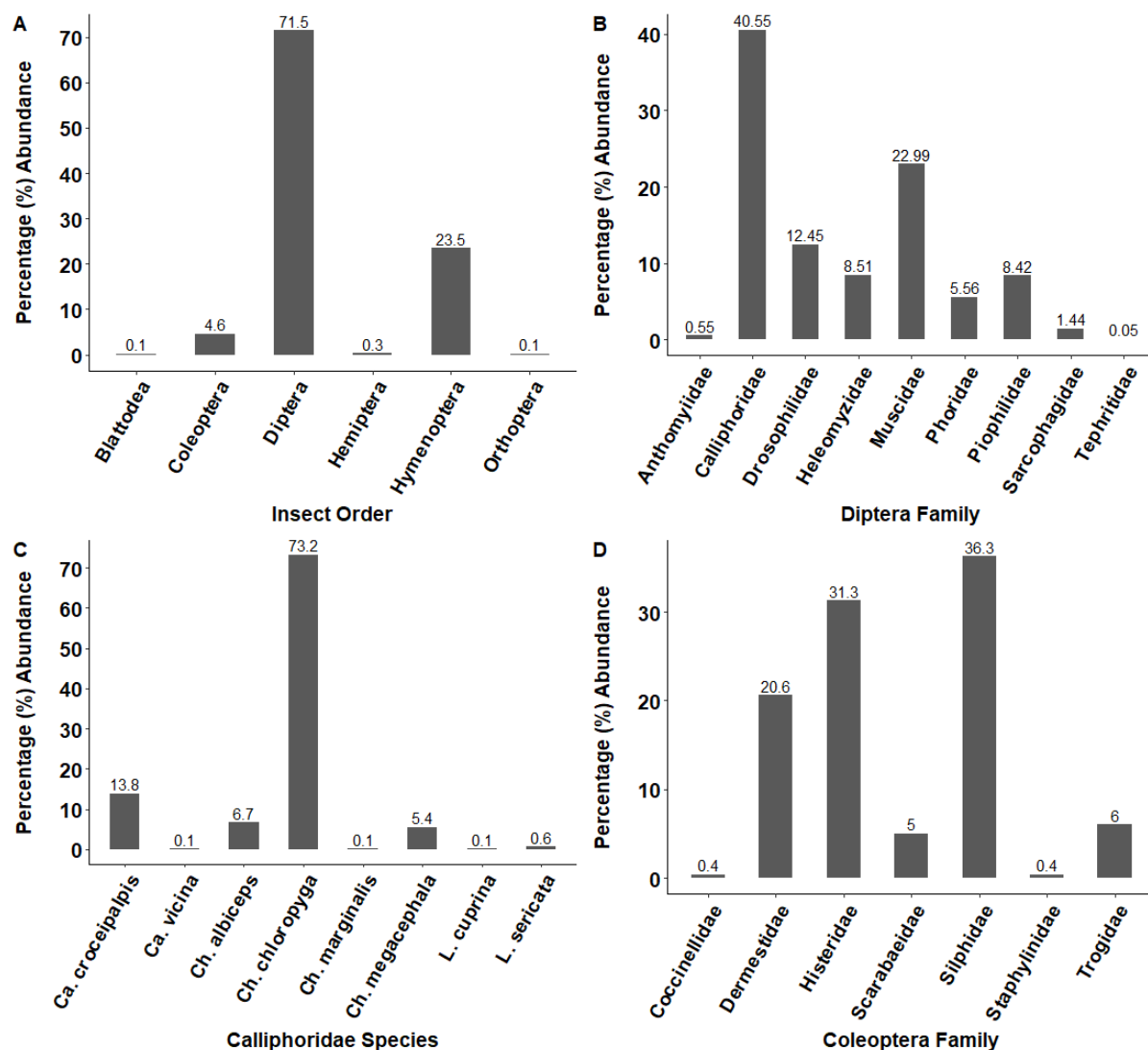


Figure 3.33: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the late winter neonate pig trial.

3.4.4. Insect diversity and abundance across various decomposition stages

In this winter season study, the number of insect species in relation to the decomposition stages significantly differed in the early (Likelihood Ratio $\chi^2 = 12.4$; DF = 3; $p = 0.006$), mid- (Likelihood Ratio $\chi^2 = 104.2$; DF = 3; $p < 0.0001$), and late (Likelihood Ratio $\chi^2 = 210$; DF = 3; $p < 0.0001$) winter trials. In the three winter trials (apart from the early winter), the advanced decomposition and skeletonization stages attracted significantly higher numbers of insect species ($p < 0.05$) when compared to other stages of decomposition (Figures B36A, B37A, B38A). Similarly, there was a statistically significant difference in the total number of insect individuals with respect to the decomposition stages in the early (Likelihood Ratio $\chi^2 = 178.4$; DF = 3; $p < 0.0001$), mid- (Likelihood Ratio $\chi^2 = 1665.1$; DF = 3; $p < 0.0001$), and late

(Likelihood Ratio $\chi^2 = 2759$; DF = 3; $p < 0.0001$) winter trials. The advanced decomposition and skeletonization stages attracted the highest number of insect individuals ($p < 0.05$) in the three winter trials (Figures B36B, B37B, B38B).

Insect diversity and abundance across various decomposition stages (combined data)

Combining the results obtained from the three trials within the winter season, the number of insect species differed significantly in relation to the decomposition stages (Likelihood Ratio $\chi^2 = 12.4$; DF = 3; $p = 0.006$). After a post-hoc Tukey test, the advanced decomposition and skeletonization stages were found to attract significantly higher number of insect species ($p < 0.05$) when compared to the fresh and early decomposition stages (Figure 3.34). With respect to the sampling months, there was no statistically significant difference in the number of insect species (Likelihood Ratio $\chi^2 = 5.4$; DF = 2; $p = 0.07$). However, there was a statistically significant interaction in the number of insect species between the decomposition stages and sampling months (Likelihood Ratio $\chi^2 = 44.9$; DF = 6; $p < 0.0001$). Pertaining to the total number of insect individuals across the three monthly trials within the season, there was a statistically significant difference in relation to the decomposition stages (Likelihood Ratio $\chi^2 = 178.4$; DF = 3; $p < 0.0001$). Across the four decomposition stages in all the three trials, the advanced decomposition and skeletonization stages attracted the highest number of insect individuals ($p < 0.05$) (Figure 3.35A). The total number of insect individuals differed significantly (Likelihood Ratio $\chi^2 = 26.5$; DF = 2; $p < 0.0001$) across the three sampling trials. After a post-hoc Tukey test, the total number of insect individuals in the late winter trial was significantly higher ($p < 0.05$) than that of the early and mid-winter trials (Figure 3.35B). Furthermore, there was a significant interaction in the total number of insect individuals between the stages of decomposition and sampling trials (Likelihood Ratio $\chi^2 = 374.5$; DF = 6; $p < 0.0001$).

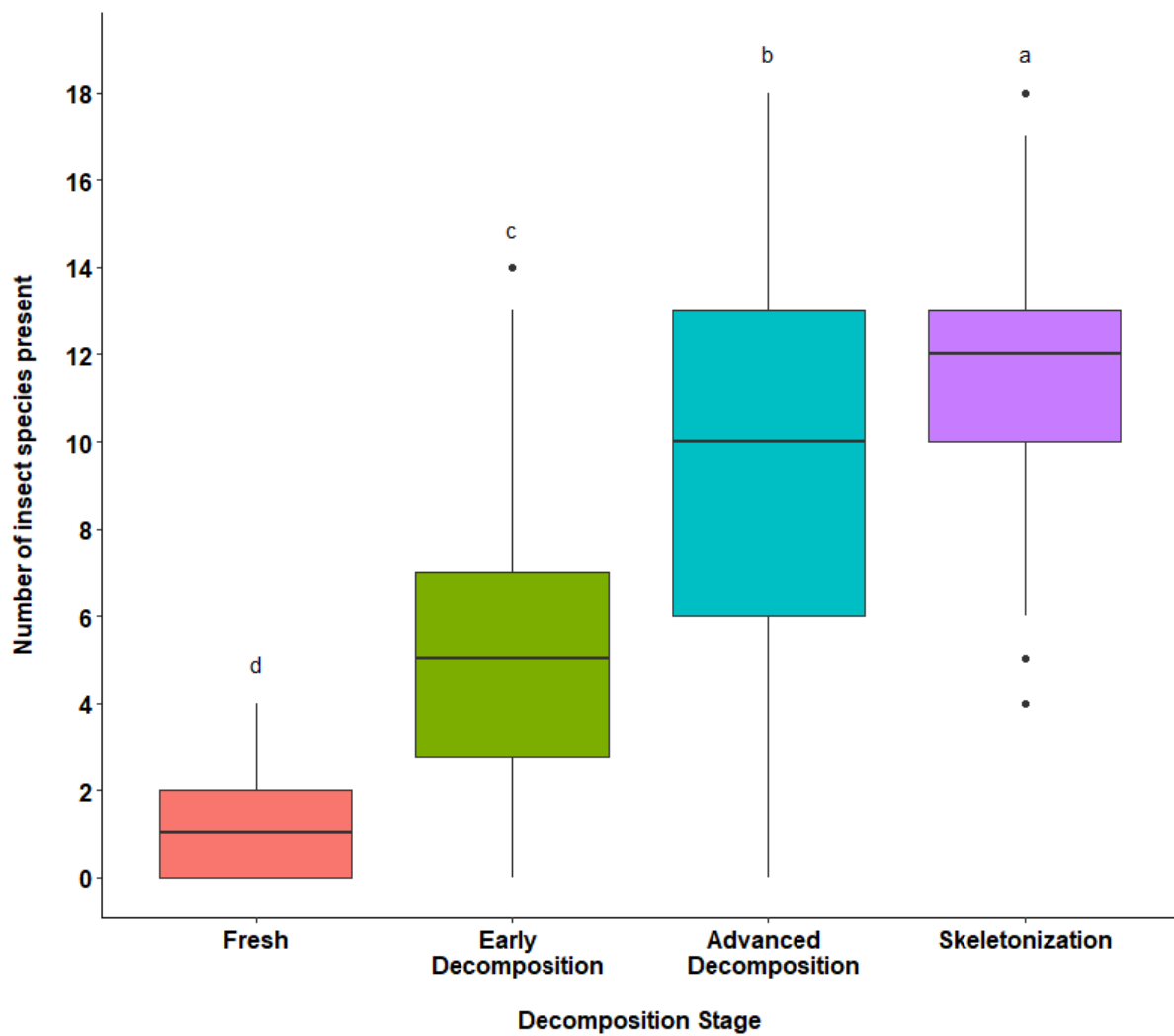


Figure 3.34: Box plot comparing the number of insect species present across the four decomposition stages in all the three neonate pig winter seasonal trials. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

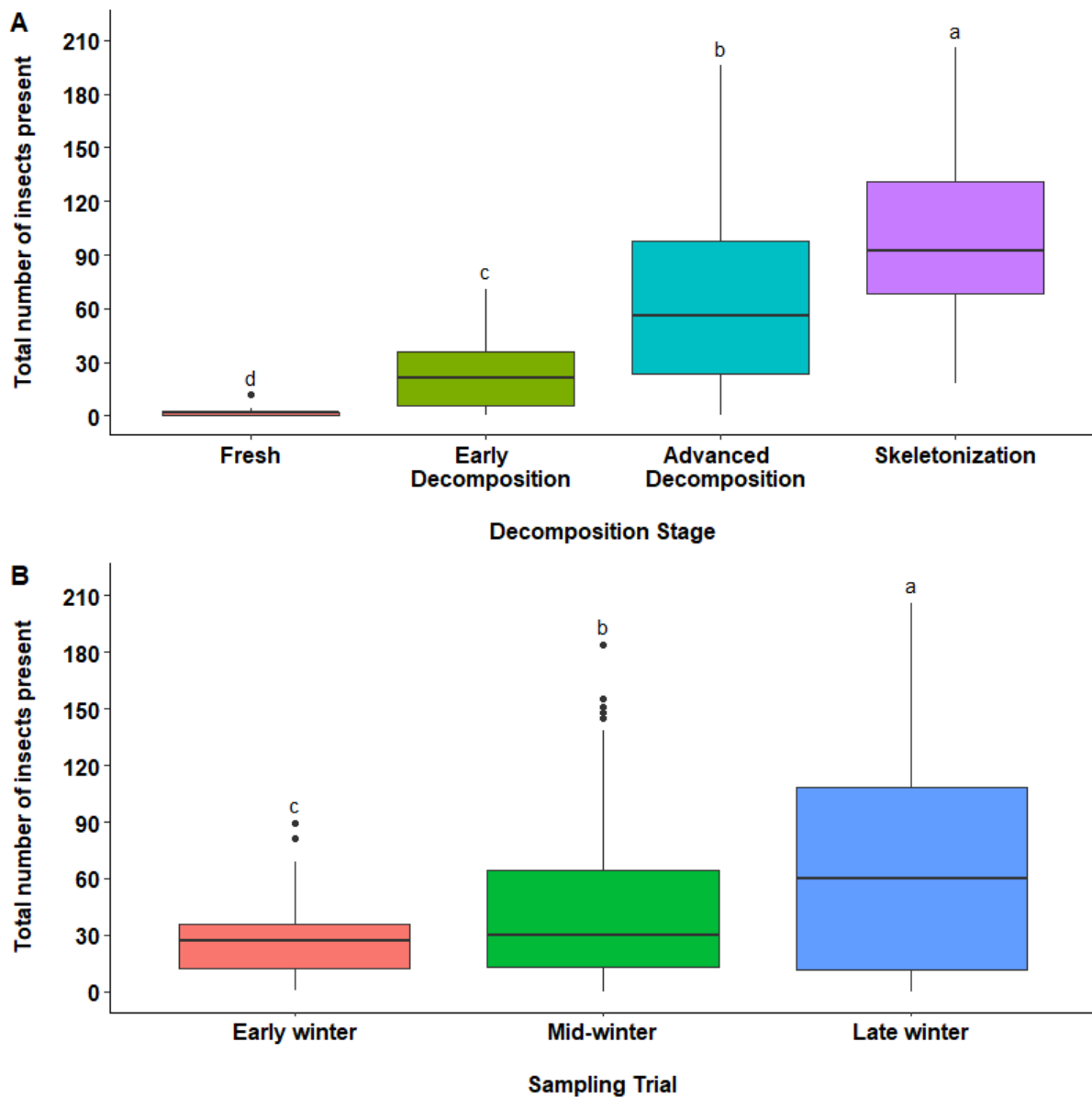


Figure 3.35: Box plots comparing the total number of insect individuals present across the four decomposition stages in all the three neonate pig winter seasonal trials (A) and total number of insect individuals present across the three winter trials (B). Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

3.4.5. Insect succession

Diptera succession

Within the family Calliphoridae, *Ca. croceipalpis* was one of the first Calliphorid flies to be present, arriving on the carcasses between Days 2 and 15 while the carcasses were in the fresh and early decomposition stages across the three winter seasonal trials (Figures B39 – B44). As from Days 5 to 25, mostly during the early and advanced decomposition stages of the carcasses, other Calliphorid flies such as *Ch. chloropyga*, *Ch. albiceps*, *H. fernandica*, *Ca. vicina*, *L. sericata*, *L. cuprina* and *Ch. marginalis* began to arrive on the carcasses in varying abundance across the three winter seasonal trials (Figures B39 – B44). In addition, some of these Calliphorid flies persisted on the carcasses until the skeletonization stage (Figures B39 – B44).

In the early winter trial, the eggs of *Ca. vicina* were recorded in the area between the forelimbs of one of the carcasses as early as Day 1, while in the mid-winter trial, the eggs of *Ca. vicina* were recorded on the left hindlimb of one of the carcasses on Day 38 (Figures B40 & B41). In the early winter trial, the adults of this Calliphorid fly were not recorded on the same carcass on which their eggs were found all through the trial. Furthermore, in the mid-winter trial, only one individual of *Ca. vicina* was recorded, arriving on the same carcass on Day 48 (10 days after its eggs were first observed) (Figure B41). It is possible that they may have accessed the carcasses earlier during the trials (which allowed for oviposition) but were only trapped later (in the case of the mid-winter trial). The eggs and first instar larvae of *Ca. croceipalpis* were recorded in the ears and oral cavities of the carcasses between Days 3 and 11 in the early and late winter trials (Figures B40, B43 and B44). In the mid-winter trial, the eggs and first instar larvae of *Ch. chloropyga* were recorded on the right forelimb of one of the carcasses on Days 32 and 34 respectively, while in the late winter trial, they were noticed in the area around the forelimbs of one of the carcasses between Days 25 and 27 (Figures B41 & B44).

Across the three winter seasonal trials, members of the family Sarcophagidae were observed to arrive on the carcasses between Days 2 and 22 (Figures B39 – B44). The first instar larvae of this family were recorded on the right forelimb of one of the carcasses (Day 32) in the mid-winter trial (Figure B41), with no larvae recorded on the carcasses in the early and late winter trials. *Musca* sp. and *Hydrotaea* sp. arrived on the carcasses mostly during the early and advanced decomposition stages across the three winter trials, and they were able to persist on the carcasses until the skeletonization stage (Figures B39 – B44). As with the earlier seasonal

trials, the colonization patterns of other Dipterous flies belonging to the families Piophilidae, Sepsidae, Anthomyiidae, Curtonotidae, Drosophilidae, Ulidiidae, Heleomyzidae, Tephritidae, Scathophagidae, and Phoridae varied considerably across the decomposition stages of the carcasses in the three winter trials (Figures B39 – B44).

Coleoptera succession

Members of the family Tenebrionidae and Scarabaeidae were the first beetles to be recorded, arriving on the carcasses as early as Days 1 and 2 in the early and late winter trial (Figures B40, B43, and B44). As the carcasses progressed into the mid-phase of early decomposition and then, advanced decomposition stages across the three winter trials, members of the families Histeridae, Silphidae, Dermestidae Carabidae, Staphylinidae, and Scarabaeidae arrived on the carcasses (Figures B39 – B44). Members of some other Coleopteran families such as Trogidae and Coccinellidae were mostly recorded on the carcasses during the skeletonization stage (Figures B40 & B44). Amongst all the beetles that were recorded on the carcasses, only the larvae of Silphid beetles were recorded on the pig carcasses and this was in the early, and mid-winter trials (Figures B39 – B42).

Hymenoptera succession and other insect orders succession

In the early and mid-winter trials, *Crematogaster cf. liengmei* was observed to arrive on the carcasses between Days 1 and 3, while in the late winter trial, they were noted on the carcasses on Day 11 (Figures B39 – B44). Following their arrival on the carcasses, they were observed to maintain a constant presence until the skeletonization stage (Figures B39 – B44). In the mid-winter trial, specifically between Days 4 and 6, the presence and feeding activities of *Crematogaster cf. liengmei* on the flesh around the limbs and upper abdominal area of the carcass resulted in the creation of bitemarks and skin lesions (Figures 3.36A – 3.36D). On Day 7, blood was seen oozing out from one of the ant-inflicted lesions on the abdominal region (Figure 3.36D). Furthermore, several individuals of *Crematogaster cf. liengmei* were seen preying and scavenging on the larval and adult stages of Calliphorid flies in the early and mid-winter trials respectively (Figures 3.37A & 3.37B).

Across the three winter trials, *A. manducator* was observed to arrive on the carcasses between Days 11 and 17 (early decomposition stage), after which they maintained a constant presence until the skeletonization stage (Figures B39 – B44). Members of other insect families and orders such as Apidae, Blattodea, Orthoptera, Hemiptera, and Mantodea were present on

the carcasses albeit in varying degrees of abundance across the three trials winter (Figures B39 – B44).

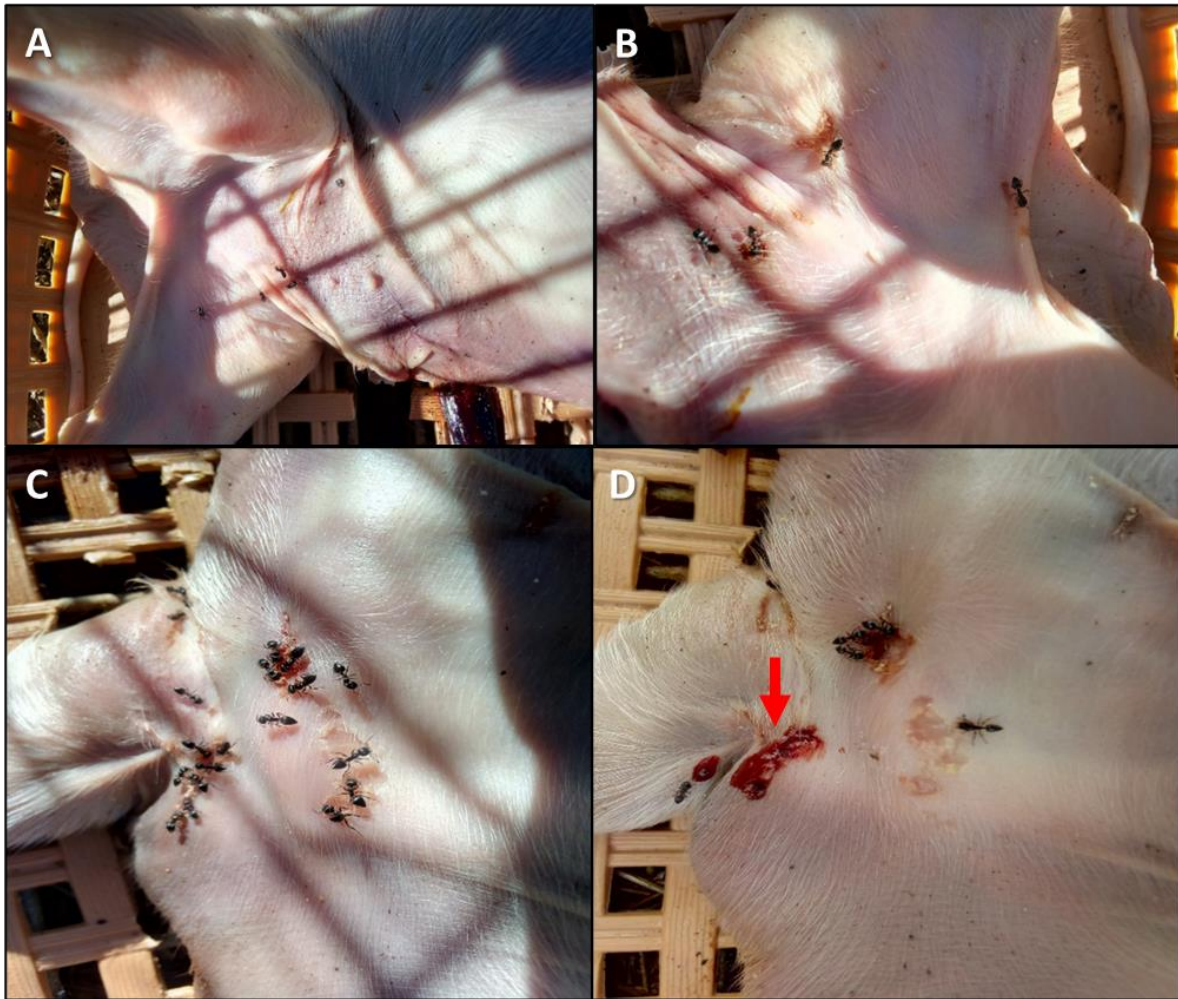


Figure 3.36: Individuals of *Crematogaster cf. liengmei* aggregating, feeding, and creating fleshy lesions between the hindlimbs and forelimbs of the carcass on Day 4 and 6 respectively during the mid-winter trial (A, B, and C). A hemorrhage (red arrow) observed from one of the lesions between the forelimbs on Day 7 (D) (Adapted from Adetimehin *et al.*, 2022).



Figure 3.37: An individual *Crematogaster cf. liengmei* carrying a fly larva away from the carcass in the early winter trial (A). Several individuals of *Crematogaster cf. liengmei* scavenging on the adult of a Calliphoridae fly in the mid-winter trial (B) (Adapted from Adetimehin *et al.*, 2022).

3.5. Discussion

In this study, the diversity, abundance, and successional patterns of insect species associated with decomposing neonate pig carcasses in each month across the four seasons (i.e., spring, summer, autumn, and winter) within the Table Mountain region of the Western Cape Province were investigated.

Impact of freezing and thawing of carcasses on the rate of decomposition, time of colonization and succession of insects

In the literature, some concerns exist with respect to the effects of freezing and subsequent thawing of a carcass on the rate of decomposition, time of arrival/colonization and successional pattern of insects (see Micozzi, 1986). However, previous studies have shown that the freezing and subsequent thawing of carcasses and skeletal muscle tissues had no significant effect on the decomposition rate, time of arrival/colonization or pattern of insect succession and vertebrate scavengers on the carcasses (Payne, 1965; Archer, 2004; Stokes *et al.*, 2009; Bugajski *et al.*, 2011; Šulaková *et al.*, 2014).

Also, some concerns exist with respect to the presence or absence of the external and internal micro-flora that triggers decomposition in stillborn pigs (Micozzi, 1986; Roberts and Dabbs, 2015). However, reports exist on the transmission of microbes from the mother to the offspring before, during and after birth in both vertebrates and invertebrates (Funkhouser and Bordenstein, 2013). Furthermore, studies have shown that there are no significant differences in microbial communities between fresh and frozen human and animal remains (Johnson *et al.*, 2013; Ogbanga *et al.*, 2023). It is noteworthy to state that several studies have utilized stillborn pig carcasses (including frozen-thawed carcasses) in conducting diverse forms of studies in forensic entomology (Payne, 1965; Archer, 2004; Cruise *et al.*, 2018; Munro *et al.*, 2019; Blonar and Prada-Tiedemann, 2020; Ziogos *et al.*, 2023).

Insect diversity and abundance across various decomposition stages

Generally, considerable differences were found in the first insect colonizers of the decomposing pig carcasses in all the trials across the four seasons. The late stages of decomposition (i.e., advanced decomposition and skeletonization) attracted the highest number of insect species and individuals across the four seasons. This is plausibly due to the opening of the carcasses alongside the robust amount of volatile organic compounds being released from the carcasses during the late stages of decomposition (Dekeirsschieter *et al.*, 2009).

Insect species assemblage and succession across the four seasons

Diptera

Of the family Calliphoridae, *Ca. croceipalpis* was one of the first colonizers, arriving on the neonate pig carcasses during the early stages of decomposition across the four seasons. The eggs and larvae of *Ca. croceipalpis* were seen on the pig carcasses in the spring and winter seasons. This blow fly is a cold-loving species prevalent in the winter season of South Africa (Prins, 1982; Richards *et al.*, 2009a, b). Its occurrence in the spring season, and specifically, in the early spring period (i.e., September) is not surprising as it has been documented as such (Prins, 1982; Richards *et al.*, 2009a, b). Despite this species' preference for the winter seasonal period, its sporadic presence across the late spring, summer, and autumn seasonal periods may be attributed to the gradual increment in ambient temperature.

While it was not expected to collect *Ca. croceipalpis* in the late spring, summer, and autumn seasonal periods, its presence within the study site may be attributed to its preference for higher altitudes (Richards, 2007). This may be supported by the fact that the study site is approximately 90 – 100 meters above sea level. In some similar studies in other Provinces (including the Western Cape) in South Africa, *Ca. croceipalpis* was either not present or documented to oviposit on decomposing pig carcasses (Kolver, 2009; Kelly *et al.*, 2011; Gilbert, 2014; Finaughty, 2019; Tembe and Mukatirawa, 2021). However, in the Transvaal Highveld which spans across the Free State and Gauteng Provinces, the immature and adult stages of *Ca. croceipalpis* were recorded on small animal carcasses (Meskin, 1986). This further validates the findings of other authors that have reported considerable differences in the community of forensically important insects between one region/locality and another within the same country (Cruise *et al.*, 2018; Díaz-Aranda *et al.*, 2018).

Calliphora vicina arrived on the carcasses mostly during the early and advanced decomposition stages in the spring and winter seasonal trials. This observation is surprising as they are often reported as one of the first colonizers of decomposing pig carcasses in the cold seasonal periods (Šulaková and Barták, 2013; Finaughty, 2019). In this study, the eggs and larvae of *Ca. vicina* were recorded on the pig carcasses specifically in the spring and winter seasons. However, the adult and immature stages of *Ca. vicina* were entirely absent on the pig carcasses in the summer and autumn seasons. As it is common with its congener *Ca. croceipalpis*, *Ca. vicina* is a cold-loving blow fly prevalent mostly in the winter season of South Africa and other countries (Richards, 2007; Battan Horenstein *et al.*, 2007; Kelly *et al.*, 2011;

Prado e Castro *et al.*, 2012; Finaughty, 2019) and this may be the reason why it was absent in the summer and autumn seasons. In agreement with the observations recorded in the winter seasonal trials of this study, other authors in the Free State Province found the immature stages of *Ca. vicina* on the pig carcasses deployed in the winter season (Kelly *et al.*, 2008).

Calliphora vicina is an invasive species in South Africa and it was first recorded in the country in 1965 (Williams and Villet, 2006b). Despite its invasive status and limited distribution in South Africa (Williams and Villet, 2006b), the occurrence of *Ca. vicina* in the Western Cape Province and specifically on the Table Mountain indicates that it is now widespread in its distribution range in South Africa, as first suggested by Finaughty (2019). The fact that the immature stages of *Ca. vicina* were observed on the pig carcasses (as seen in the spring and winter seasonal trials) despite its low abundance, supports the previous claim by Williams and Villet (2006b) about the forensic significance of this species and its importance in the estimation of the PMImin in South Africa.

Chrysomya chloropyga was the dominant and most abundant fly and/or Calliphorid species across the spring, and winter seasons. This corroborates with the findings of other authors in the spring and winter seasons of the Free State and Western Cape Provinces (Kelly, 2006; Kolver, 2009; Kelly *et al.*, 2011; Finaughty, 2019). The eggs of this blow fly species were recorded on the pig carcasses in all the four seasons, corroborating the findings of other authors that have documented the immature stages of *Ch. chloropyga* on pig carcasses across the spring, summer, autumn and winter seasons (Kelly *et al.*, 2008, 2011). The thermal and seasonal flexibility/tolerance of *Ch. chloropyga* may be the reason why they were able to successfully deposit their eggs on the pig carcasses across the four seasons (Richards *et al.*, 2009a, b).

In this study specifically during the late spring trial, the immature stages of *Ch. chloropyga* were recorded on one of the carcasses during the advanced decomposition stage (Day 8) despite its early presence. Similar observation was also recorded on small animal carcasses by Meskin (1986). The delayed oviposition by *Ch. chloropyga* may be attributed to the early presence of non-gravid females and age of the carcass (Barbosa *et al.*, 2023), and it might pose a problem especially when immature insect evidence is to be used in time since death estimation. Owing to the presence of the immature stages of *Ch. chloropyga* on the carcasses across the four seasons, the author suggests that it is a multi-seasonal indicator of the

season of death, and care should be taken when attempting to utilize this species in the determination of the specific season of death.

Chrysomya megacephala, also known as the oriental latrine fly, is an invasive fly that was first reported in South Africa in 1971 (Williams and Villet, 2006b). According to Richards *et al.* (2009a), the distribution of *Ch. megacephala* is somewhat along the coastline in South Africa specifically in the Western Cape Province, as well as in Mpumalanga, KwaZulu-Natal, Limpopo, and Northern Free State. This restricted distribution may be connected to its preference for high humidity (Richards *et al.*, 2009a). *Chrysomya megacephala* was one of the first Calliphorid species to colonize the pig carcasses, often arriving during the fresh and early decomposition stages across the four seasons. This blow fly species was found to be the most abundant and dominant fly in the summer season, and second most abundant Calliphorid species in the autumn season. These observations are consistent with the findings of Shi *et al.* (2009) in China. However, it differs from the findings of other authors who showed *Ch. marginalis* and *Ch. albiceps* respectively to be the dominant Calliphorid species in the summer season of the Free State and Gauteng Provinces of South Africa (Kelly *et al.*, 2011; Gilbert, 2014).

Across the four seasonal trials, the immature stages of *Ch. megacephala* were only recorded on the pig carcasses in the summer season. Therefore, it is not out of place to state that it is an indicator of the summer season when their immature life stages (e.g., eggs, larvae and pupae) are found on decomposing vertebrate remains within the Province. The occurrence of *Ch. megacephala* in this study, differs from the findings of other similar studies in South Africa across multiple seasons where the insect species was not recorded on decomposing pig and sheep carcasses (Kelly *et al.*, 2008, 2009, 2011; Kolver, 2009; Gilbert, 2014; Botham, 2016; Finaughty, 2019; Tembe and Mukatirawa, 2021, Tembe *et al.*, 2021a, b). The absence of *Ch. megacephala* on decomposing vertebrate remains in other Provinces of South Africa may be attributed to its invasive status and restricted coastal distribution (Williams and Villet, 2006b; Richards *et al.*, 2009a, b). Thus, this study is the second to report the presence of the adult and immature stages of *Ch. megacephala* on decomposing vertebrate remains in the Western Cape after Prins (1982). Furthermore, this study is possibly the first to empirically report the colonization pattern, and successful oviposition of *Ch. megacephala* on decomposing carcasses in South Africa across multiple seasons. Owing to this it is suggested that *Ch. megacephala* could potentially be used as an indicator of body translocation between Provinces in South Africa.

Chrysomya albiceps was the second most abundant Calliphorid fly recorded across the spring, summer, and winter seasons. However, in the autumn season, it was the most abundant. The abundance of *Ch. albiceps* across multiple seasons has been reported by several authors in South Africa and elsewhere (e.g., Battan Horenstein *et al.*, 2007; Abd El-Bar and Sawaby, 2011; Kelly *et al.*, 2011; Gilbert, 2014; Shayya *et al.*, 2018a, b; Sebastião and Prado e Castro 2019; Zeariya and Kabadaia, 2019; Finaughty, 2019; Tembe and Mukaratirwa, 2021). The immature stages of this blow fly were recorded on the pig carcasses only in the summer season despite its consistent occurrence and dominance in other seasons. *Chrysomya albiceps* is known to prefer warmer temperatures and this may explain why it was able to successfully oviposit on the pig carcasses in the summer season.

In this study, *Ch. marginalis* was the least abundant *Chrysomya* species recorded across the four seasons. Similar observations were reported in the summer season of Luanda, Angola (Sebastião and Prado e Castro, 2019). However, other authors in the Free State Province reported *Ch. marginalis* to be the most abundant fly in the summer season (Kelly *et al.*, 2011). Furthermore, a study within the Kruger National in Limpopo reported the abundance of *Ch. marginalis* on decomposing impala carcasses in the summer season (Braack, 1986). In this study, the immature stages of *Ch. marginalis* were recorded on one of the carcasses, but only during the summer season. This observation may be attributed to its preference for warmer temperatures (Richards *et al.*, 2009a, b; Sebastião and Prado e Castro, 2019). Generally, the low abundance of *Ch. marginalis* across the four seasons might be linked to its preference for the remains of large vertebrates (Braack, 1984; Meskin, 1986; Sebastião and Prado e Castro, 2019). Nevertheless, the presence of the larvae of *Ch. marginalis* on one of the carcasses in the summer season suggests its usefulness in PMImin estimations and as an indicator of the summer season within the Western Cape Province.

Hemipyrellia fernandica was one of the Calliphorid flies found on the pig carcasses across the four seasons. The immature stages of this blow fly species were recorded on the pig carcasses in the summer and autumn seasons. This is consistent with the findings of Meskin (1986) in the Transvaal Highveld. The presence and successful oviposition of *H. fernandica* on the pig carcasses in the summer and autumn seasonal trials of this study may be attributed to its preference for smaller-sized carcasses and the summer/autumn seasons (Meskin, 1986; De Jong and Krell, 2011). This is in agreement with the findings of Zumpt and Ledger (1967) who reported the presence of several *H. fernandica* maggots in a small wound on the back of a live Cape Hegdehog in Bryanston near Johannesburg, South Africa. In the published successional

data from other Provinces across several seasons in South Africa within the past three decades, both the adult and immature stages of this Calliphorid fly were not reported on decomposing vertebrate remains (Kolver, 2009; Kelly *et al.*, 2011; Gilbert, 2014; Finaughty, 2019; Tembe and Mukaratirwa, 2021; Tembe *et al.*, 2021a, b). However, the adult and immature stages of this blow fly species were recorded on small animal carcasses in the Transvaal Highveld which spans across the Free State and Gauteng Provinces (Meskin, 1986).

As with *Ch. megacephala*, *H. fernandica* is a forensically significant Calliphorid fly in the Western Cape Province that can provide useful information relating to the minimum time since death. Their restricted distribution within the country suggest that they could be used in the verification of body translocation between Provinces in South Africa. The abundance of other Calliphorid flies such as *L. sericata*, *L. cuprina* and *Pericallimyia* sp., was very low and this may be due to their preference for sub-urban, rural, and thicketed/forested habitats respectively (Zumt, 1956; Meskin, 1986). It is noteworthy to state that *Pericallimyia* sp. is a relatively new Calliphorid fly that has rarely been reported on decomposing carcasses. Notwithstanding, Setyaningrum and Al Dhafer (2014), were able to collect a few adult individuals of a congener, *Pericallimyia greatheadi* (Zumt, 1971), using baited traps in Saudi Arabia. To the best of the author's knowledge, this study is likely the first to report the presence of this genus/species on decomposing carcasses in Africa. Owing to this, its forensic importance should be further explored.

Members of the family Sarcophagidae were one of the first colonizers of the carcasses across the four seasons, arriving sometimes before or after the Calliphorid and Muscid flies. This is consistent with the findings of other similar studies (Vasconcelos *et al.* 2013; Cruise *et al.*, 2018; Maisonhaute and Forbes, 2021; Tembe and Mukaratirwa, 2021). Members of this family were generally present on the carcasses from the fresh to the late stages of decomposition, and this is in line with the findings of other similar studies (Abajue *et al.*, 2014; Castro *et al.*, 2019). In contrast, other authors have noted the presence of Sarcophagid flies in the bloated, active, and advanced decomposition stages (Kelly *et al.*, 2011; Shayya *et al.*, 2018b).

In this study, the number of adult Sarcophagids was generally low when compared to the Calliphorids and Muscids and this corroborates with the findings of other authors (Mabika *et al.*, 2014; Kelly *et al.*, 2011; Shayya *et al.*, 2018b; Finaughty, 2019; and Sebastião and Prado e Castro, 2019) but contradicts the findings of Vasconcelos *et al.* (2013). The low abundance

of Sarcophagid adult flies could be linked to the inherent competition with the Calliphorid flies (Shi *et al.*, 2009; Cruise *et al.*, 2018; Castro *et al.*, 2019). Nevertheless, Sarcophagid flies are known to larviposit (i.e., lay their larvae) on decomposing carcasses, and this gives their larvae a developmental and resource exploitation lead over the larvae of other fly species (Cruise *et al.* 2018). The larvae of Sarcophagid flies were recorded on the carcasses in all the four seasons. In the Free State Province, specifically in the winter season, the immature stages of Sarcophagid flies have also been documented on decomposing pig carcasses (Kolver, 2009; Kelly *et al.*, 2011). In some trials (e.g., mid-winter trial), delayed larviposition by Sarcophagid flies was observed and this may be linked to either the non-gravid status of the early arriving female flies, age of the carcass, or low temperatures of the winter season (Prado e Castro *et al.*, 2012; Barbosa *et al.*, 2023). Above all, the fact that the larvae of Sarcophagid flies were recorded on the pig carcasses in all seasons, validates their importance as a forensically significant insect family that can be used in the estimation of the PMIm in across multiple seasons in the Western Cape Province.

Members of the family Muscidae were observed to arrive on the neonate pig carcasses at the same time, and sometimes after the arrival of Calliphorid and Sarcophagid flies in several trials across the four seasons. These colonization patterns have also been reported by several authors (Buchan and Anderson, 2001; Byrd and Castner, 2010; Amendt *et al.*, 2011; Kelly *et al.*, 2011). In this study, *Musca* sp. was the most abundant Muscid fly, however, they did not deposit their eggs on the carcasses in all the four seasons. This is consistent with the findings of other similar studies across several seasons (Kelly *et al.*, 2011; Aly *et al.*, 2017; Shayya *et al.*, 2018b; Castro *et al.*, 2019; Sebastião and Prado e Castro, 2019; Hu *et al.*, 2023). Despite the early presence of *Musca* sp. on some of the carcasses, it is not unusual to state that it only came to feed on the exudates from the carcasses.

Across the four seasons, *Hydrotaea* sp. was present on the pig carcasses during the late phase of the early decomposition stage and predominantly during the advanced decomposition and skeletonization stages. This is consistent with the findings of other authors who reported the presence of several adult *Hydrotaea* species during the advanced decomposition stage of an exposed pig carcass in Southern Italy (Bonacci *et al.*, 2021). In agreement with Byrd and Castner (2010), *Hydrotaea* sp. was seen frequently within the fly nettings hovering around the carcasses and sometimes directly above the steel cages. As with *Musca* sp., *Hydrotaea* sp. did not deposit its eggs on the carcasses, and this is consistent with the findings of Kelly *et al.* (2011) and Hu *et al.* (2023). *Neomyia viridescens* was one of the third Muscid fly reported in

this study and was only found in the spring and autumn seasons. This species is predominantly a dung-breeding fly and empirical information on its ecology is scarce (Wall *et al.*, 2008). The rare presence of *N. viridescens* on the decomposing carcasses as seen in this study might be attributed to its preference for animal dung and not animal remains (Wall *et al.*, 2008).

Suillia picta belonging to the family Heleomyzidae was recorded on and around the pig carcasses in the spring, autumn and winter seasons. However, in the summer season, it was entirely absent. While they are known to be carrion feeders (Picker *et al.*, 2019), their abundance was generally low when compared to the Calliphorid and Muscid flies. According to Soszyńska-Maj and Woźnica (2016), members of the family Heleomyzidae are cold-adapted species active in the winter season. This may explain why they were not collected during the summer season in this study. Despite their low abundance, the general presence of *S. picta* within the study site may be attributed to its preference for higher altitudes (Soszyńska-Maj and Woźnica, 2016). This study may be the first to report the presence of both *N. viridescens* and *S. picta* on decomposing carcasses in Africa. Owing to their presence on the decomposing pig carcasses as shown in this study, it is important to conduct additional studies on these two species with the aim of exploring their forensic relevance in the Western Cape Province and beyond.

Members of the family Piophilidae, Sepsidae, Phoridae and Scathophagidae were mainly present during the late phase of the early decomposition stage and the advanced/skeletonization stages across the four seasons. This is consistent with the findings of other authors across several seasons (Kelly *et al.* 2009, 2011; Boehme *et al.*, 2010; Silahuddin *et al.*, 2015; Oleksakova *et al.*, 2016; Shayya *et al.* 2018b; and Finaughty, 2019). According to Byrd and Castner (2010) and Heo *et al.* (2011), members of these families (i.e., Piophilidae and Sepsidae) are usually attracted to dry proteinaceous food materials such as carrion, bones, skin, and fur and this might explain their abundance in the late decomposition stages. Similarly, members of the family Scathophagidae are dung eaters and their abundance during the late stages of decomposition might be attributed to the exposure of the intestinal contents of the carcasses (Byrd and Castner, 2010).

It is assumed that the low abundance and limited presence of the Phorid flies on exposed decomposing carcasses is linked to their preference for carcasses buried or in confined environments (Disney, 2008; Reibe and Madea, 2010; Zuha *et al.*, 2017). Other Dipteran families such as Drosophilidae, Anthomyiidae, Tephritidae, and Ulidiidae were also recorded

in this study and they have been reported to be present on different types of decomposing vertebrate carcasses in several studies (Disney, 2008; Byrd and Castner, 2010; Ekanem and Dike, 2010; Dupont *et al.*, 2011; Prado e Castro *et al.*, 2012; Mabika *et al.*, 2014; Abajue *et al.*, 2014; Abd El-Gawad *et al.*, 2016; Shayya *et al.*, 2018b).

Hymenoptera

The forensic importance of several ant species in the process of carrion decomposition and successional patterns of carrion-associated insects has begun to gain the attention of forensic entomologists (Eubanks *et al.*, 2019; Adetimehin *et al.*, 2022). Across the spring, summer, autumn and winter seasonal trials, members of the family Formicidae were recorded on and around the pig carcasses. This observation is consistent with the findings of several other authors in the literature (Eubanks *et al.*, 2019). In the spring, autumn, and winter seasons, several individuals of the ant species, *Crematogaster* cf. *liengmei*, were found gathering and feeding on the flesh of the carcasses specifically in the area around and between the limbs, abdominal regions, and body orifices (i.e., eyes, mouth, nose, and ears) (Adetimehin *et al.*, 2022). Their voracious feeding activities around these regions resulted in the creation of bite marks, and multiple skin lesions on the flesh of the pig carcasses (Adetimehin *et al.*, 2022). In the spring season, the intense feeding of these ants prevented blow flies from landing on one of the carcasses, and the deposition of their eggs and/or establishment of larval masses (Adetimehin *et al.*, 2022).

In the winter season, blood was seen oozing out from one of the ant-inflicted skin lesions on one of the carcasses (Adetimehin *et al.*, 2022). To the best of the author's knowledge, this is the first time such will be reported in Africa. The skin lesions and the resultant discharge of blood observed in this study may be attributed to the action of the chemical secretions produced by ants on the epidermal layer of the carcass skin during the process of feeding (Adetimehin *et al.*, 2022). Equally important, in the autumn season, some of the ant-inflicted lesions on one of the carcasses were exploited by blow flies as suitable sites for oviposition (Adetimehin *et al.*, 2022). This is the second time such observation would be recorded in the global forensic entomology literature with the first being the findings of Meyer *et al.* (2020). However, this study is the first in Africa to document such observations. Apart from the summer season, several individuals of *Crematogaster* cf. *liengmei* were seen on multiple occasions, preying on the eggs and larvae of flies, and also scavenging on the remains of Calliphorid flies (Adetimehin *et al.*, 2022).

The parasitic wasp, *A. manducator* (Braconidae) arrived on the carcasses across the four seasons mostly during the early and advanced decomposition stages of the pig carcasses. Generally, the arrival of this parasitic wasp species was often in synchrony with the occurrence of fly eggs and larvae on the carcasses. Furthermore, the abundance of this species was gradually observed to increase following oviposition and/or larviposition by blow flies and flesh flies. In the spring and winter seasons, several *A. manducator* were seen aggregating around the eggs and preying on the larvae of Calliphoridae and Sarcophagids flies. In contrast, no predatory and parasitic behavior of *A. manducator* on blow fly and flesh fly larvae were observed in the summer and autumn seasons. Members of the same group (i.e., wasps) including those belonging to the family Braconidae have been reported to prey on and parasitize fly eggs, larvae, pupae, and adults (Adetimehin *et al.*, 2023a) and the forensic implications of the parasitic and predatory behavior of parasitoid wasps including *A. manducator* are sufficiently detailed in Adetimehin *et al.* (2023a).

Coleoptera

Towards the late phase of early decomposition and early phase of advanced decomposition, the carcasses began to show some signs of dryness and bone exposure especially around the head, neck, and limbs. These stages have been documented to be attractive to a different cohort of insects collectively known as beetles (Byrd and Castner, 2010). In the warm seasonal periods (i.e., summer and autumn seasons), members of the family Dermestidae and Histeridae were the most abundant and this is consistent with the findings of other authors in South Africa and elsewhere (Kelly *et al.* 2011; Mabika *et al.*, 2014; Sebastião and Prado e Castro, 2019; Ries *et al.*, 2021).). However, in the cooler seasonal periods (spring and winter seasons) members of the family Silphidae were the most abundant. This contradicts the findings of other authors in the Free State Province who found *Dermestes maculatus* (Dermestidae) to be the most abundant beetle species on pig carcasses in the winter season (Kolver, 2009; Kelly *et al.*, 2011).

Members of the family Dermestidae have been reported to prefer dried carcasses and this might explain the reason behind their abundance in the late stages of decomposition (Abd El-Bar *et al.* 2016; Ries *et al.*, 2021). As the carcasses transitioned into the advanced decomposition stages, the majority of fly larvae migrated deep into the oral cavities, forming several masses within the upper region of trunk and sometimes underneath the carcasses. Due to their combined necrophagous and predatory behavior, members of the families Histeridae, Staphylinidae, Silphidae, and Scarabaeidae were abundant during the advanced decomposition

and skeletonization stages and were found in close association with blow fly and flesh fly larvae feeding in and on the tissues underneath the abdominal regions of the carcasses.

The occurrence of the individuals belonging to the family Carabidae in the autumn season differed from the findings in the summer and spring seasons where they were not recorded. Similarly, the occurrence of few individuals belonging to the family Cleridae in the summer season differed from the findings of the spring, autumn and winter seasons where they were not observed on or around the pig carcasses. This observation was surprising, and it significantly differed from the findings of other authors in the Free State and Western Cape Provinces who recorded numerous individuals of *Necrobia rufipes* (De Geer, 1775) during the advanced decomposition stage of pig carcasses in the summer season (Kelly *et al.*, 2009; Finaughty, 2019).

The presence of several individuals of the families Histeridae, Silphidae, Staphylinidae, Scarabaeidae, Cleridae, and Dermestidae during the early and late stages of decomposition corroborates with the findings of other similar studies (Braack, 1987; Anderson and Vanlaerhoven, 1996; Magni *et al.*, 2019; Griffiths *et al.*, 2020; Tembe and Mukaratiwa, 2021). Amongst these Coleopteran families recorded, only the larvae of Silphid (spring, autumn and winter seasons) and Dermestid (spring season) beetles were recorded on the pig carcasses. This is consistent with the results of other authors across the spring, autumn and winter seasons in the Free State and Western Cape Provinces (Kelly *et al.*, 2009, 2011; Finaughty, 2019).

Conclusion

In summary, this study has revealed the insect species that can be used not only in the estimation of the PMImin on a monthly temporal scale, but also, as indicators of specific seasons when found on decomposing human remains. In the spring season for instance, *Ch. chloropyga*, *Ca. vicina*, *Ca. croceipalpis*, and members of the families Sarcophagidae, Silphidae and Dermestidae are the most important insect species as their immature stages were recorded on the pig carcasses. In the summer season, *Ch. megacephala*, *Ch. albiceps*, *Ch. marginalis*, *Ch. chloropyga* and *H. fernandica* are the most forensically significant insect species owing to the presence of their immature stages on the pig carcasses. In the autumn season, *Ch. chloropyga*, *H. fernandica* and members of the families Sarcophagidae and Silphidae are the most important insect species due to the reason above. Lastly, in the winter season, the forensically important insect species include *Ch. chloropyga*, *Ca. vicina*, *Ca. croceipalpis*, and members of the families Sarcophagidae, Silphidae. Above all, it is worth mentioning that the immature stages

of some species such as *Ch. chloropyga* and Sarcophagidae gen. sp. were recorded on the pig carcasses across the four seasons. Therefore, caution should be employed when attempting to use them as indicators of a specific season of death.

Chapter 4: Results and Discussion – Adult Pig Decomposition and Insect Succession Trials

4.1. Summer Season

4.1.1. Weather data

In this trial, daily mean external carcass temperature ranged between 21.4°C to 30.3°C (overall mean = 24.9°C) (Figure 4.1A). Daily mean ambient temperature ranged between 17.8°C to 24.4°C (overall mean = 20.7°C) (Figure 4.1A). Daily mean ambient relative humidity ranged between 54.6% to 93.6% (overall mean = 68.4%), while daily rainfall ranged between 0mm to 15mm (overall mean = 0.9mm) (Figure 4.1B).

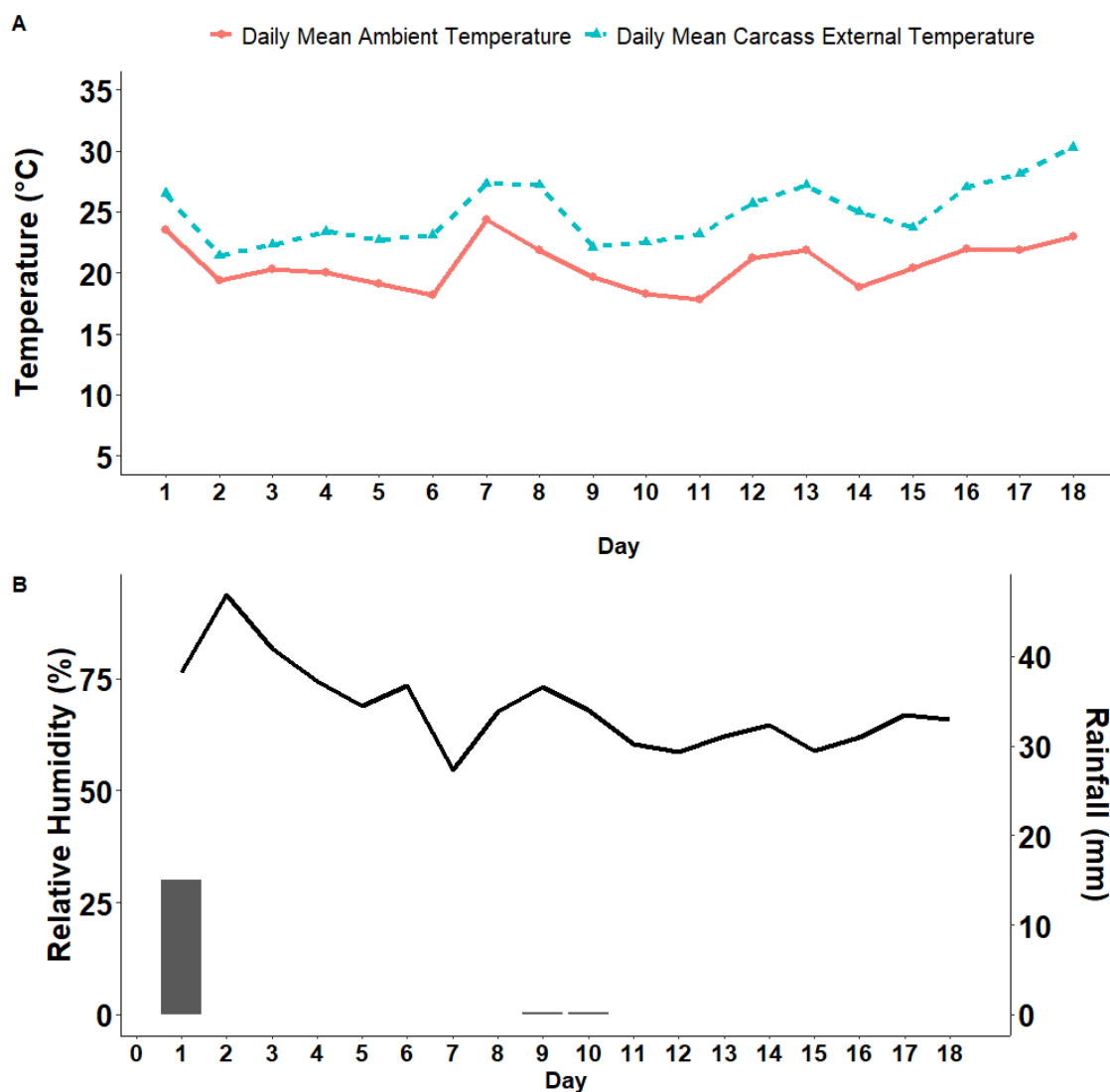


Figure 4.1: Daily mean ambient, and external carcass temperatures (A), and daily mean ambient relative humidity (line) and rainfall (bars) (B) during the adult pig summer trial.

4.1.2. Decomposition data

Fresh Stage (TBS = 3): This stage commenced immediately after the termination of the pig and the deployment of its carcass within the study site. Following termination and deployment in the study site, no significant changes in the coloration of the carcass were observed (Figure 4.2A). This stage was observed to last for a period equal to or less than 1 Day ($ADD \leq 26.5$).

Early Decomposition Stage (TBS: 4 – 16): This stage was marked by the light to dark-red change in the coloration of the skin around the head, neck, and limbs. The abdominal region of the carcass had a glossy appearance. Slight inflammation of the head, neck and abdominal regions of the carcass was noted during the early phase, and this inflammation (especially around the abdominal region) increased as decomposition progressed (Figure 4.2B). There was a significant increase in the presence and activities of different adult insects (i.e., flies and ants) in, on and around the carcass, especially within the oral and nasal cavities, in and around the ears, and between the fore- and hindlimbs. Freshly laid egg masses and newly emerged larvae of blow flies were also observed in these regions. Due to increased feeding intensity and abundance of blow fly larvae during the late phase of early decomposition, the carcass began to show some signs of skin slippage, formation of blisters and hair loss (Figures 4.3A & 4.3B). More decomposition fluids began to seep from the head and neck, and the abdominal regions into the underlying and surrounding soil. Furthermore, these decomposition fluids together with some third instar blow fly larvae were observed to flow downhill in a south-westerly direction from the carcass (Figure 4.3C). The “larval migration stream” was 1.5m long and 40cm wide. At the termination point, the “larval migration stream” tapered to a width of 17cm following which the larvae began to disperse randomly (see Supplementary Video C1). This stage began on Day 1 ($ADD = 26.5$) and lasted up to Day 5 ($ADD = 116.2$).

Advanced Decomposition Stage (TBS: 17 – 24): This stage was marked by the caving of the head and neck and deflation of the trunk. Large quantities of decomposing fluids were seen oozing out from the abdominal region into the underlying and surrounding soil. Furthermore, the carcass began to show some signs of extensive skin slippage, and bone exposure in the area around the head and neck, and limbs. Blow fly larvae were all over the body, but the majority were concentrated in the upper and lower abdominal regions as well as the area between the limbs (Figure 4.2C). During the late phase of advanced decomposition, the majority of the flesh in the head and neck region was consumed. Furthermore, the intestinal contents of the carcass became visible, and a significant number of larvae were observed migrating from the carcass in the western direction. However, there was a considerable reduction in the number of larvae

migrating from the carcass through the downhill flowing decomposition fluids. This decomposition stage was short-lived as it was observed to begin on Day 6 (ADD = 139.4) and end on Day 7 (ADD = 166.7).

Skeletonization Stage ($TBS \geq 25$): This stage was characterized by the darkening and drying out of the carcass skin, loss of intestinal contents, significant flattening of the abdominal region coupled with increased bone exposure and some greasy substances (Figure 4.2D). The number of larvae feeding on the carcass were also observed to have significantly reduced. During this stage, what was left of the carcass were the bones, some thin and mummified skin, and dry hairs. This stage commenced on Day 8 (ADD = 193.8) and the experimental trial was terminated on Day 18 (ADD = 448.6).



Figure 4.2: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of the carcass (A – D) during the adult pig summer trial.



Figure 4.3: Massive larval activity and feeding in the blisters on the upper region of the trunk and forelimbs (A), right hindlimb (B), and the downhill and southwesterly migration of blow fly larvae together with the decomposition fluids on the 5th Day of the adult pig summer trial (C).

4.1.3. Abundance and diversity of identified insect taxa

A total of 2,132 adult insect individuals belonging to four orders (Diptera, Hymenoptera, Coleoptera, and Hemiptera) were recorded. Amongst the insect orders recorded, the order Diptera (68.15%) was the most abundant followed by the orders Hymenoptera (15.9%), Coleoptera (15.85%), and Hemiptera (0.1%) (Figure 4.4A). Within the order Diptera, a total of 1,453 individuals were recorded of which members of the family Calliphoridae (42.7%) were the most abundant followed by the families Muscidae (27.7%), Piophilidae (26.2%), Sarcophagidae (3.4%), and Anthomyiidae (0.1%) (Figure 4.4B). Within the family Calliphoridae, a total of 620 Calliphorid flies were recorded of which *Ch. chloropyga* (55.3%), was the most abundant followed by *Ch. albiceps* (31.6%), *Ch. megacephala* (8.7%), *L. cuprina* (2.1%), and fewer individuals (<1%) of *L. sericata*, *H. fernandica*, *Ch. marginalis*, and *Ca. croceipalpis* (Figure 4.4C). Amongst the order Coleoptera, a total of 338 individuals of which the members of the family Histeridae (43.5%) were the most abundant, followed by the Dermestidae (35.2%), Cleridae (9.5%), Silphidae (8.3%), and fewer individuals (<4%) of the families Tenebrionidae and Staphylinidae (Figure 4.4D).

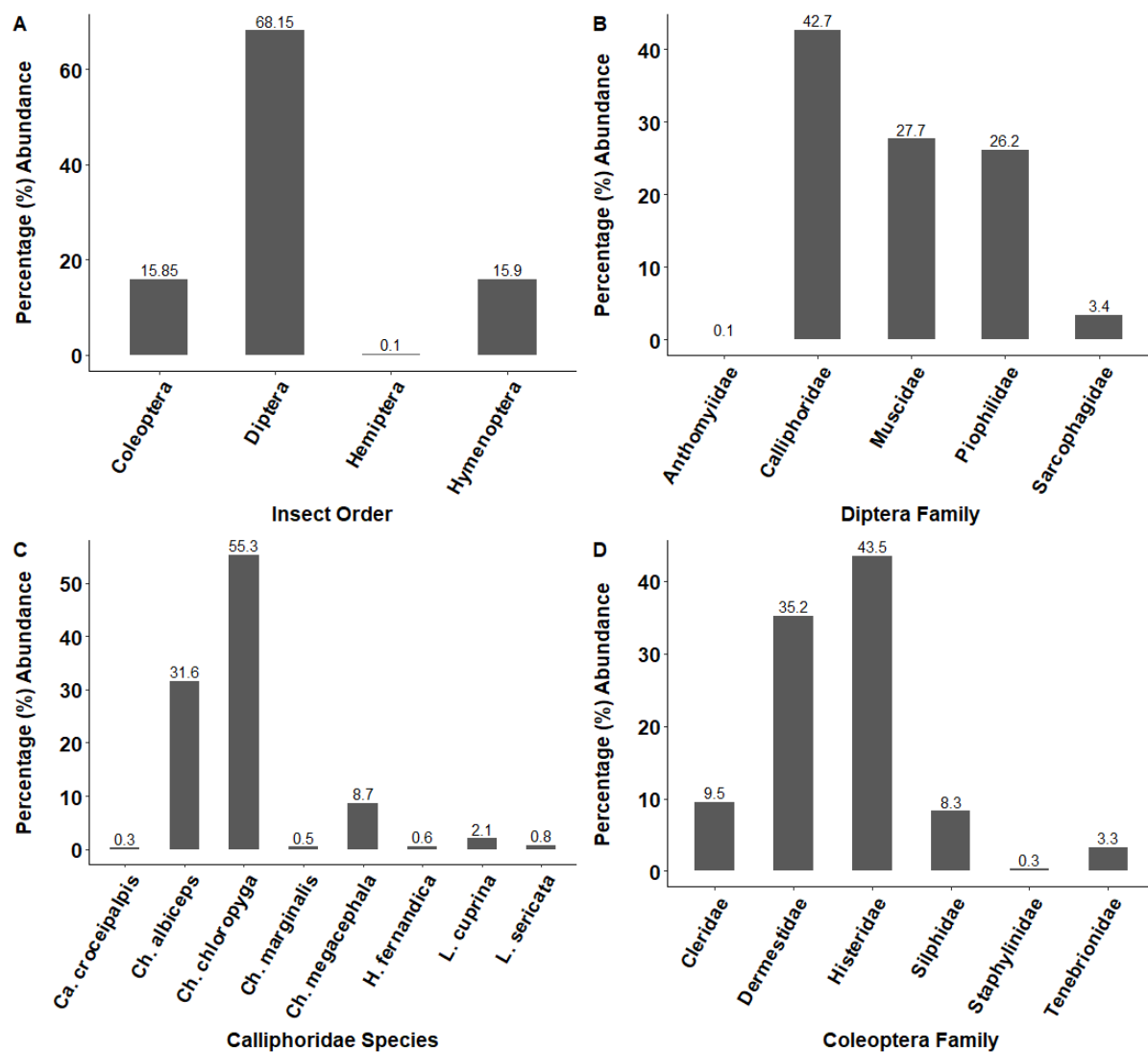


Figure 4.4: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the adult pig summer trial.

4.1.4. Insect diversity and abundance across various decomposition stages

In this trial, the number of insect species in relation to the decomposition stages significantly differed (Likelihood Ratio $\chi^2 = 14.07$; DF = 3; $p = 0.003$). Furthermore, a significantly higher number of insect species ($p < 0.05$) were attracted to the carcass during the early decomposition, advanced decomposition and skeletonization stages than when the carcass was fresh (Figure 4.5A). However, no significant difference ($p > 0.05$) was observed in the number of insect species across the early decomposition, advanced decomposition and skeletonization stages (Figure 4.5A). There was a statistically significant difference in the total number of insect individuals with respect to the decomposition stages (Likelihood Ratio $\chi^2 = 325.8$; DF = 3; $p < 0.0001$). A significantly higher number of insects ($p < 0.05$) were recorded during the advanced decomposition and skeletonization stages when compared to the fresh and early decomposition stages (Figure 4.5B).

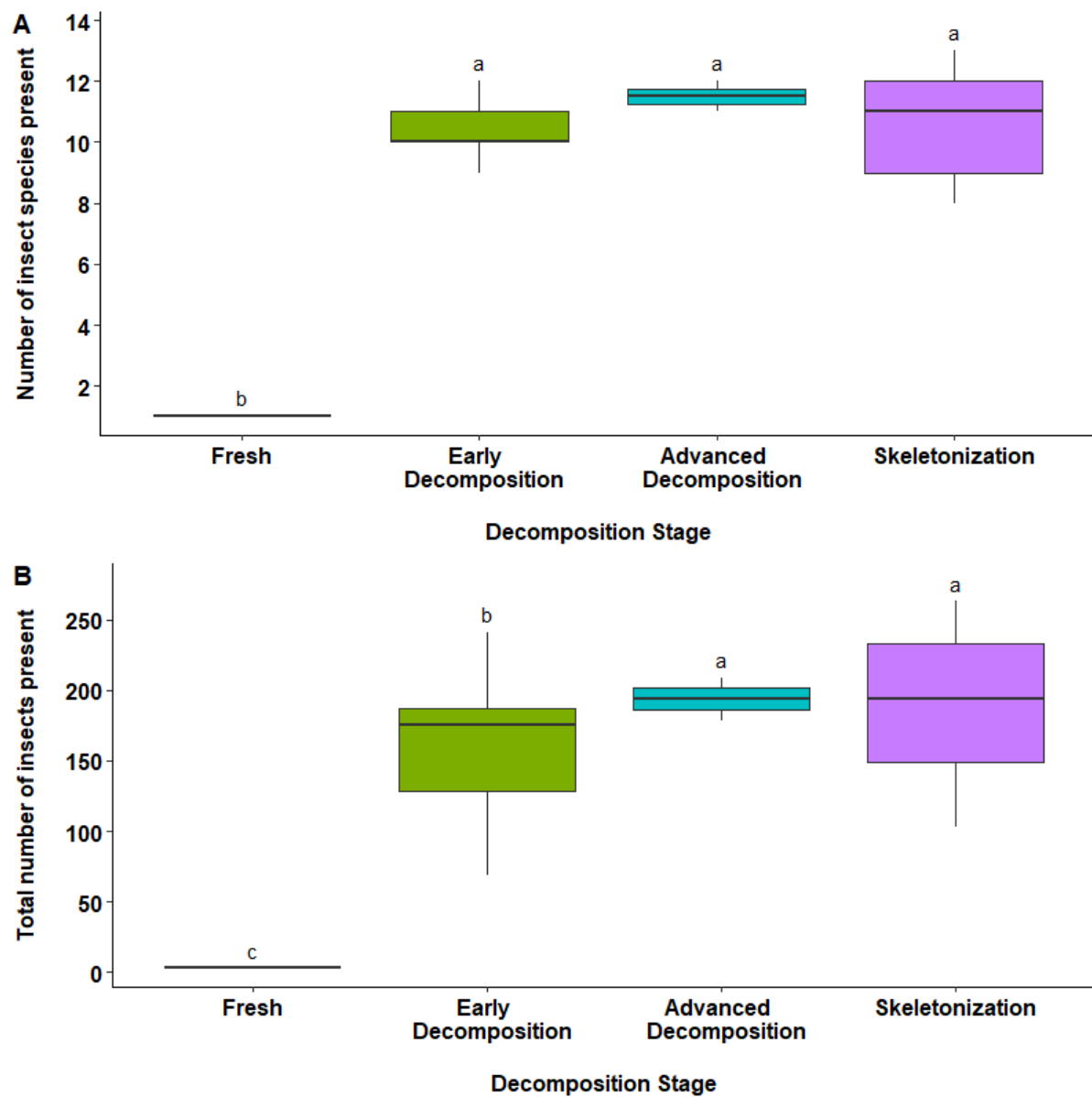


Figure 4.5: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the adult pig summer trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

4.1.5. Insect succession

Diptera succession

Within the family Calliphoridae, *Ch. megacephala*, *Ch. chloropyga*, *H. fernandica*, and *Ca. croceipalpis* were the first colonizers, arriving and interacting with the pig carcass as early as Day 1 following carcass deployment and onset of early decomposition (Figure 4.6). Other Calliphorid flies such as *Ch. albiceps*, *L. sericata*, and *L. cuprina*, were observed to arrive on the carcass on Day 2, while *Ch. marginalis* arrived on Day 3 (Figure 4.6). Among these blow fly species, only *Ch. megacephala*, *Ch. chloropyga*, *Ch. albiceps*, and *L. cuprina* persisted on the pig carcass until the onset of the skeletonization stage albeit in varying abundance.

Freshly laid egg masses of *Ch. chloropyga* were recorded in the oral cavity and area around the right ear of the pig carcass on Day 2 (Figure 4.6). More egg masses and first instar larvae of *Ch. chloropyga* were recorded in the area around the left ear and right hind limb of the pig carcass on Days 4 and 5 respectively (Figure 4.6). First and second instar larvae of *Ch. albiceps* were recorded on the abdominal region, area around and behind the head/neck of the pig carcass and in the decomposition fluids, together with the larvae of *Ch. chloropyga*, oozing out of the pig carcass and flowing downhill in the southwestern direction on Days 4 and 5 (Figure 4.6).

Adult members of the family Sarcophagidae were recorded on the pig carcass as early as Day 1, and they persisted until the skeletonization stage (Figure 4.6). *Musca* sp. was the first responder, arriving within 30 minutes following the deployment of the pig carcass (Figure 4.6). This Muscid fly was present on the pig carcass from the fresh stage of decomposition to the skeletonization stage. In contrast, *Hydrotaea* sp., arrived towards the end of the early decomposition stage (Day 4) and persisted until the skeletonization stage (Figure 4.6). Members of the family Piophilidae arrived on the carcass during the late phase of early decomposition (Day 5) and were present on the carcass until the skeletonization stage. Members of the family Anthomyiidae were scanty in abundance and were only observed on the pig carcass on selected days during the early decomposition (Day 1) and advanced decomposition (Day 6) stages (Figure 4.6).

Coleoptera succession

Members of the families Tenebrionidae and Staphylinidae were the first beetle colonizers, arriving on the pig carcass as early as Days 1 and 2 respectively while the carcass was in the early decomposition stage (Figure 4.6). Following the transition of the carcass to the advanced

decomposition stage on Day 6, members of the families Dermestidae, Silphidae and Histeridae were all observed to arrive on the carcass, while members of the family Cleridae arrived a day after (i.e., Day 7) (Figure 4.6). Members of all these beetle families persisted on the carcass until the skeletonization stage in varying numbers except for the family Staphylinidae. Only the larvae of Dermestid beetles were seen on the carcass, and this was on Day 18, during the termination stage of the experimental trial (Figure 4.6).

Hymenoptera and other insect orders succession

Crematogaster cf. *liengmei*, were one of the first colonizers of the carcass, arriving as early as Day 1 following the onset of the early decomposition stage (Figure 4.6). Individuals of this ant species were consistently observed feeding and creating tiny bite marks on the flesh between the hindlimbs and external part of the left ear of the carcass. Furthermore, they were present on and around the pig carcass from their time of arrival (i.e., Day 1) until the skeletonization stage (Day 14).

Alysia manducator was only observed on the pig carcass on Day 3, and the individuals of this wasp species were observed walking over and around the larval mass in the oral cavity (Figure 4.6). Members of the wasp family Vespidae were scanty in abundance and were only observed on the pig carcass on selected days during the early decomposition (Day 1) and skeletonization (Day 8) stages (Figure 4.6). Members of other insect orders such as the Hemiptera were limited in abundance and were only observed underneath the carcass on Day 2 (Figure 4.6).

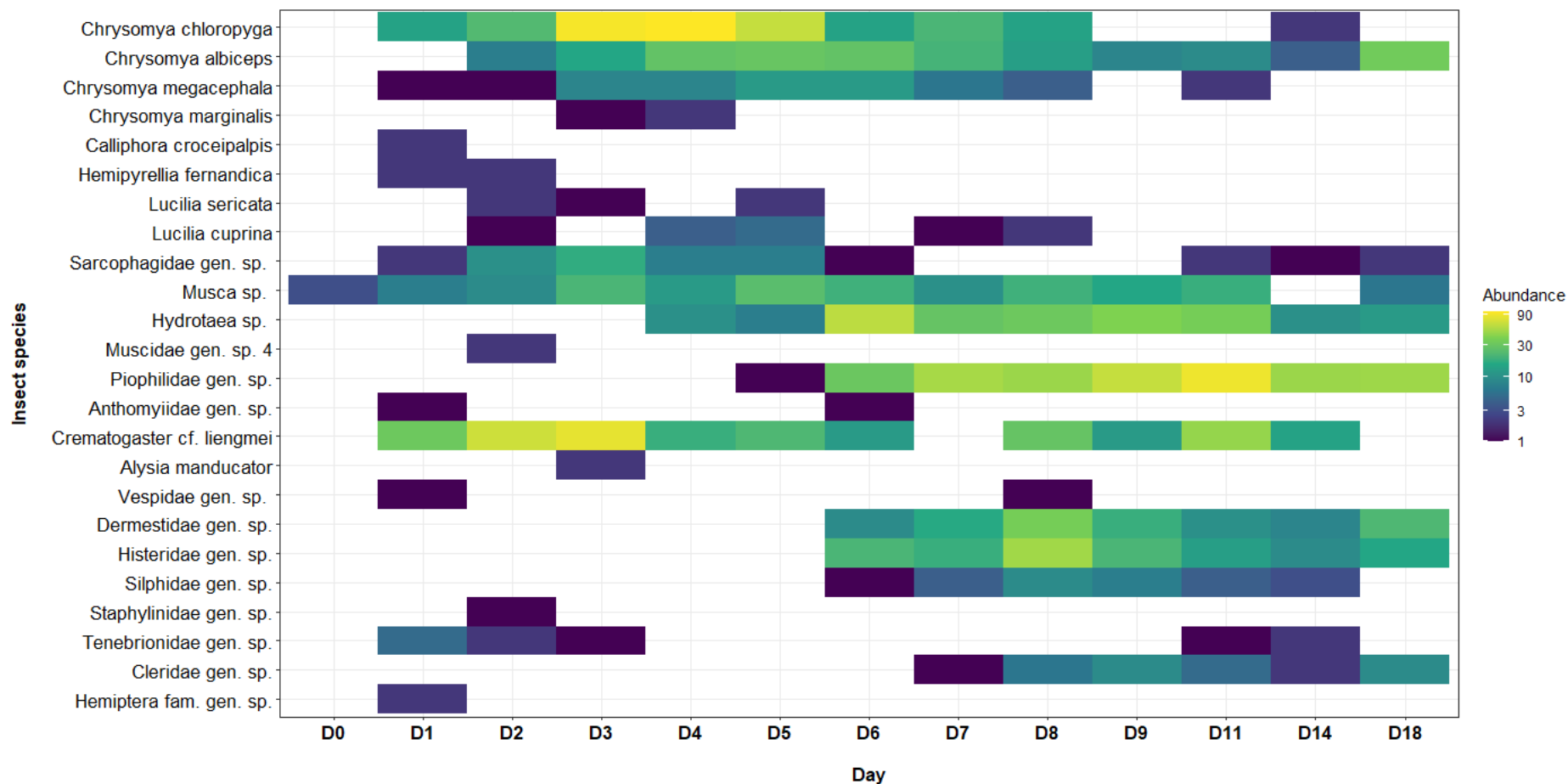


Figure 4.6: Daily abundance of adult insect species on the carcass in the adult pig summer trial. Fresh stage: D0; Early decomposition: D1 – D5; Advanced decomposition: D6 – D7; Skeletonization: D8 – D18. Eggs of *Ch. chloropyga* were recorded on Day 2; L1 and L2 larvae of *Ch. albiceps* were recorded on Days 4 and 5; Larvae of *Dermestidae gen. sp.* were recorded on Day 18.

4.1.6. Influence of carcass size on insect species and individuals

Prior to conducting outright comparisons between the data generated during the adult pig trial and those generated during the neonate pig trials, the differences in ambient weather conditions such as temperature, rainfall and relative humidity were analyzed and summarized below.

Adult pig trial vs early summer neonate pig trial

The ambient temperature (t-test = 0.9; DF = 25.5; $p = 0.85$) and rainfall ($p = 0.91$) data generated within these two different trials were not significantly different. In contrast, there was a significant difference in the ambient relative humidity (t-test = - 2.3; DF = 25.93; $p = 0.03$), with the neonate pig trial having significantly higher relative humidity when compared to the adult pig summer trial. The number of insect species differed significantly with respect to the size of the carcass (Likelihood Ratio $\chi^2 = 3.96$; DF = 1; $p = 0.046$). The adult pig carcass attracted a significantly ($p < 0.05$) higher number of insect species when compared to the neonate pig carcasses (Figure 4.7A). In contrast, carcass size had no significant influence on the total number of insect individuals attracted to the pig carcasses (Likelihood Ratio $\chi^2 = 0.79$; DF = 1; $p = 0.37$) (Figure 4.7D).

Adult pig trial vs mid-summer neonate pig trial

There were no significant differences in the ambient temperature (t-test = - 0.5; DF = 28.5; $p = 0.62$), relative humidity (t-test = - 0.49; DF = 27.65; $p = 0.63$) and rainfall ($p = 0.51$) between the two trials. There was no significant difference in the number of insect species attracted to the adult and neonate pig carcasses (Likelihood Ratio $\chi^2 = 0.85$; DF = 1; $p = 0.35$) (Figure 4.7B). However, carcass size had a significant influence on the total number of insect individuals attracted to the pig carcasses (Likelihood Ratio $\chi^2 = 28.99$; DF = 1; $p < 0.0001$). The adult pig carcass attracted significantly ($p < 0.05$) higher numbers of insect individuals when compared to the neonate pig carcasses (Figure 4.7E).

Adult pig trial vs late summer neonate pig trial

There were no significant variations in the ambient temperature (t-test = 0.15; DF = 23.66; $p = 0.88$), relative humidity (t-test = 0.03; DF = 26.75; $p = 0.98$), and rainfall ($p = 0.95$) between the two trials. The size of the carcass exerted no significant impact on the number of insect species attracted to the pig carcasses (Likelihood Ratio $\chi^2 = 0.03$; DF = 1; $p = 0.86$) (Figure 4.7C). In contrast, there was a significant difference in the total number of insects individuals with respect to carcass size (Likelihood Ratio $\chi^2 = 143.14$; DF = 1; $p < 0.0001$). The adult pig

carcass attracted significantly ($p < 0.05$) higher numbers of insect individuals when compared to the neonate pig carcasses (Figure 4.7F).

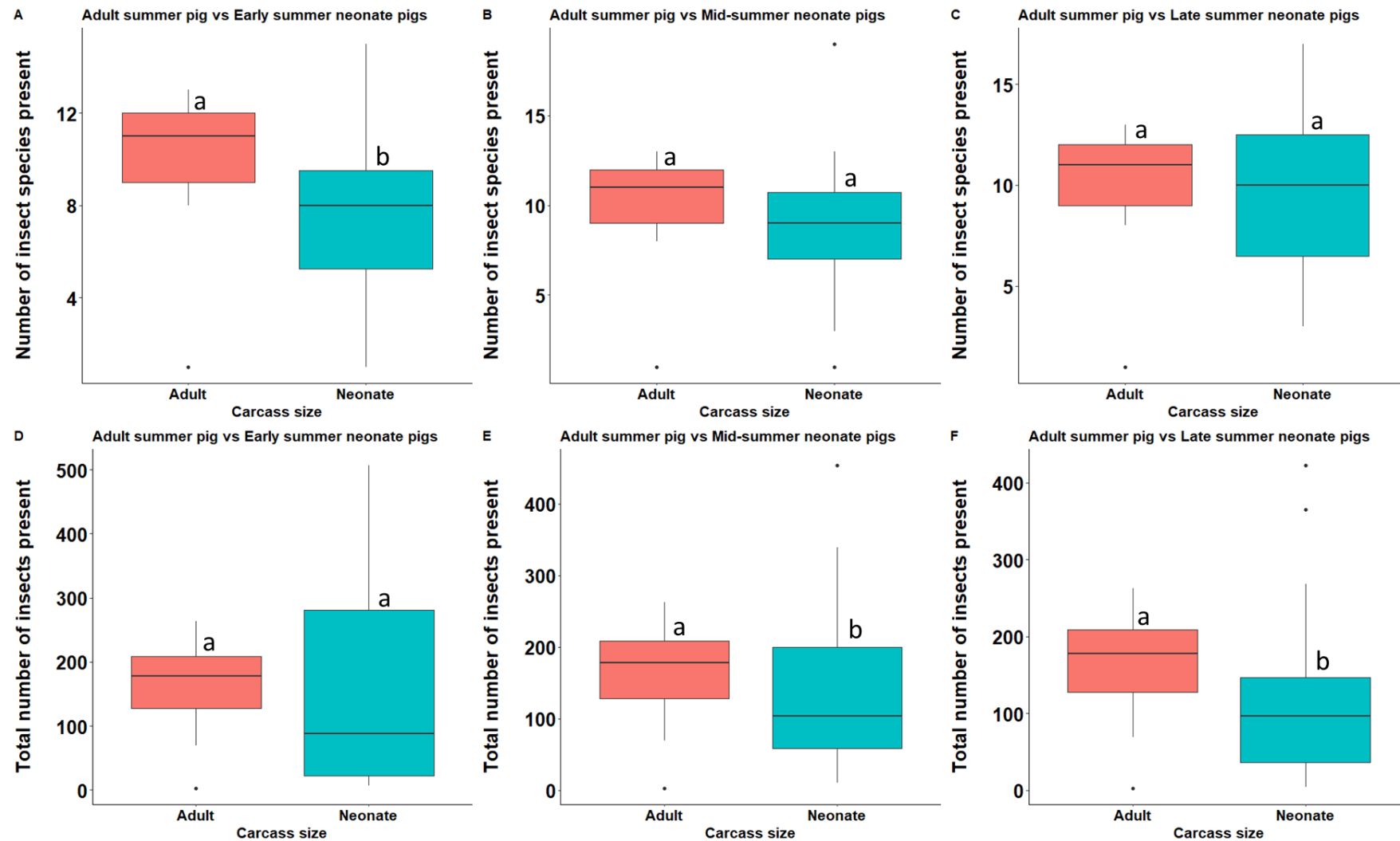


Figure 4.7: Box plots comparing the number of insect species (A – C) and total number of insects (D – E) attracted to the adult summer pig carcass and neonate pig early, mid-, and late summer carcasses. Boxes capped with different letters are significantly different after Tukey’s HSD test ($p < 0.05$).

4.2. Winter Season

4.2.1. Weather data

In this trial, the daily mean internal carcass temperature ranged between 6.7°C to 21.3°C (overall mean = 13.4°C) (Figure 4.8A). Daily mean ambient temperature ranged between 11.1°C to 23.8°C (overall mean = 17.2°C) (Figure 4.8A). Daily mean ambient relative humidity ranged between 32.5% to 96.4% (overall mean = 64.8%), while daily rainfall ranged between 0mm to 85mm (overall mean = 3.4mm) (Figure 4.8B).

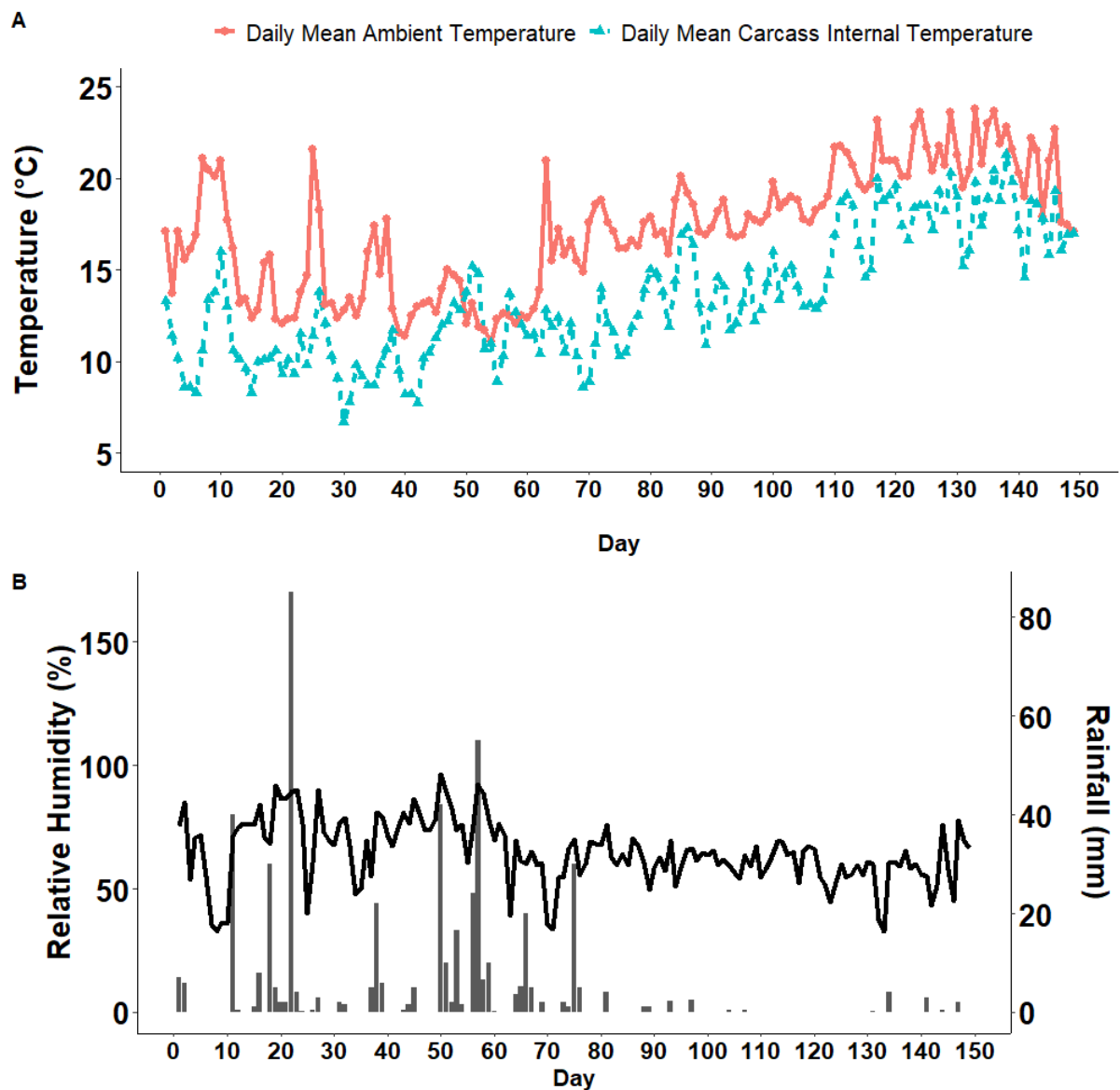


Figure 4.8: Daily mean ambient, and internal carcass temperatures (A), and daily mean ambient relative humidity (line) and rainfall (bars) (B) during the adult pig winter trial.

4.2.2. Decomposition data

Fresh Stage (TBS = 3): This stage commenced immediately after the termination and placement of the carcass within the study site (Figure 4.9A). Following termination, collection and deployment in the study site, no significant changes in the coloration of the carcass in the three scoring regions were observed. This stage was observed to end on Day 5 (ADD = 52.1).

Early Decomposition Stage (TBS: 4 – 16): This stage was marked by the glossy appearance and slight bloating of the carcass abdominal region. Inflammation of the head, neck and abdominal regions of the carcass was noted, and this became more obvious as decomposition progressed (Figure 4.9B). Owing to increased bloating, the rectum of the pig was observed protruding out of the anus. Decomposition fluids mixed with blood began to seep from the oral and nasal cavities of the carcass. The presence of certain insects such as ants alongside the feeding activities of blow fly larvae were noticeable especially within the oral and nasal cavities, in and around the ears. Freshly laid egg masses and massive larval masses of blow flies were also observed on and underneath the abdomen, around the ocular, nasal and oral cavities. Due to increased feeding intensity and abundance of blow fly larvae during the late phase of early decomposition, the carcass began to show some signs of skin slippage, and hair loss. More decomposition fluids began to seep from the head and neck area, eyes and mouth and the area around the limbs. Slight caving in of the flesh around the eyes and the upper abdominal region were also noticeable. This stage began on Day 6 (ADD = 60.5) and lasted up to Day 47 (ADD = 486.4).

Advanced Decomposition Stage (TBS: 17 – 24): This stage was marked by the caving of the head and neck and the abdomen. More decomposing fluids were seen seeping out from the ocular, nasal and oral cavities and the abdominal region into the underlying and surrounding soil. In addition, extensive skin slippage and hair loss were noticed (Figure 4.9C). Blow fly larvae were majorly concentrated in the area of the abdomen in contact with the soil. Some larvae were seen feeding on the area between the hind- and forelimbs. During the late phase of advanced decomposition, the majority of the flesh in the head and neck region was consumed. The flesh/skin around the abdominal region began to mummify with several openings. The intestinal contents of the carcass were also observed protruding out from the opening on the abdomen. This stage began on Day 48 (ADD = 499.5) and lasted up to Day 121 (ADD = 1496.2).

Skeletonization Stage (TBS ≥ 25): This stage was characterized by the mummification of the carcass skin, and complete skeletonization and decapitation of the head and neck region (Figure 4.9D). There was also a significant loss of intestinal contents and flattening of the abdominal region. The mummified and leathery skin formed a shell-like covering over the carcass. This stage commenced on Day 122 (ADD = 1512.8) and the experimental trial was terminated on Day 149 (ADD = 2002.3).



Figure 4.9: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of the carcass (A – D) during the adult pig winter trial.

4.2.3. Abundance and diversity of identified insect taxa

A total of 9,534 adult insect individuals belonging to four orders (Diptera, Hymenoptera, Coleoptera, and Blattodea) were recorded. Amongst the insect orders recorded, the order Diptera (75.1%) was the most abundant followed by the orders Coleoptera (12.9%), Hymenoptera (11.9%), and Blattodea (0.1%) (Figure 4.10A). Within the order Diptera, a total of 7,164 individuals were recorded of which members of the family Calliphoridae (54.01%) were the most abundant followed by the families Piophilidae (29.82%), Muscidae (14.29%), and other families (Anthomyiidae, Sarcophagidae, Heleomyzidae, Drosophilidae, Bibionidae, Phoridae, and Sepsidae) accounting for 1.89% (Figure 4.10B). Within the family Calliphoridae, a total of 3869 Calliphorid flies were recorded of which *Ch. chloropyga* (90.54%), was the most abundant followed by *Ch. albiceps* (5.45%), *Ca. croceipalpis* (3.31%) and fewer individuals (<1%) of *Ch. megacephala*, *Ch. marginalis*, *L. cuprina* and *Ca. vicina* (Figure 4.10C). Amongst the order Coleoptera, a total of 1,232 individuals of which the members of the family Dermestidae (40.7%) were the most abundant, followed by the Silphidae (39.3%), Histeridae (15.9%), Cleridae (2.8%), and fewer individuals (<1.5%) of the families Trogidae, Staphylinidae and Scarabaeidae (Figure 4.10D).

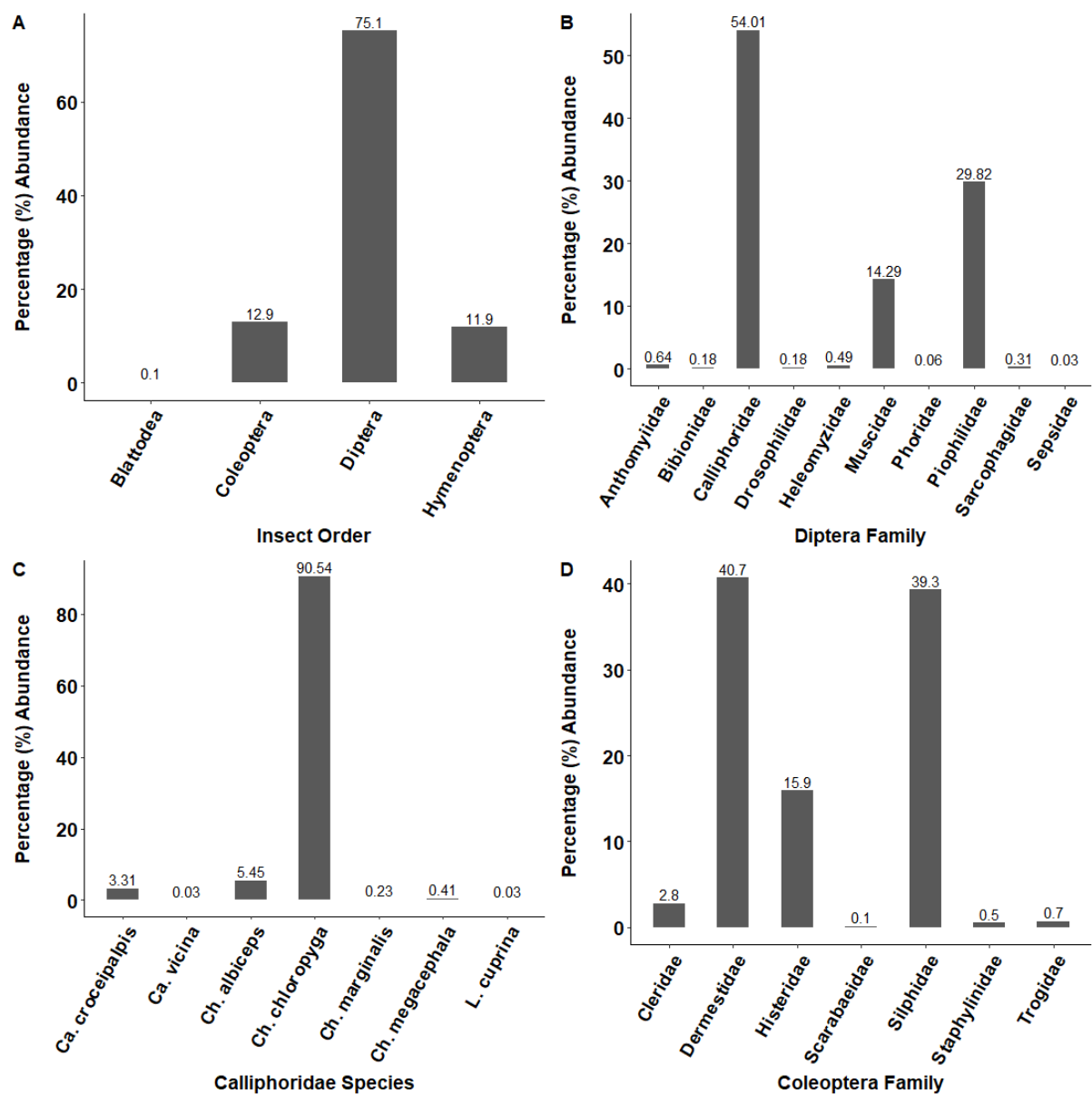


Figure 4.10: Percentage abundances of (A) Insect orders, (B) Diptera families, (C) Calliphoridae species and (D) Coleoptera families in the adult pig winter trial.

4.2.4. Insect diversity and abundance across various decomposition stages

In this trial, the number of insect species in relation to the decomposition stages differed significantly (Likelihood Ratio $\chi^2 = 31.55$; DF = 3; $p < 0.0001$). Furthermore, a significantly greater number of insect species ($p < 0.05$) were attracted to the carcass during the advanced decomposition stage when compared to other stages of decomposition (Figure 4.11A). However, no significant difference ($p > 0.05$) was observed in the number of insect species between the early decomposition and skeletonization stages (Figure 4.11A). There was a significant difference in the total number of insect individuals with respect to the decomposition stages (Likelihood Ratio $\chi^2 = 3129.1$; DF = 3; $p < 0.0001$). The advanced decomposition stage attracted the highest number of insect individuals ($p < 0.05$), followed by the early, skeletonization and fresh decomposition stages respectively (Figure 4.11B).

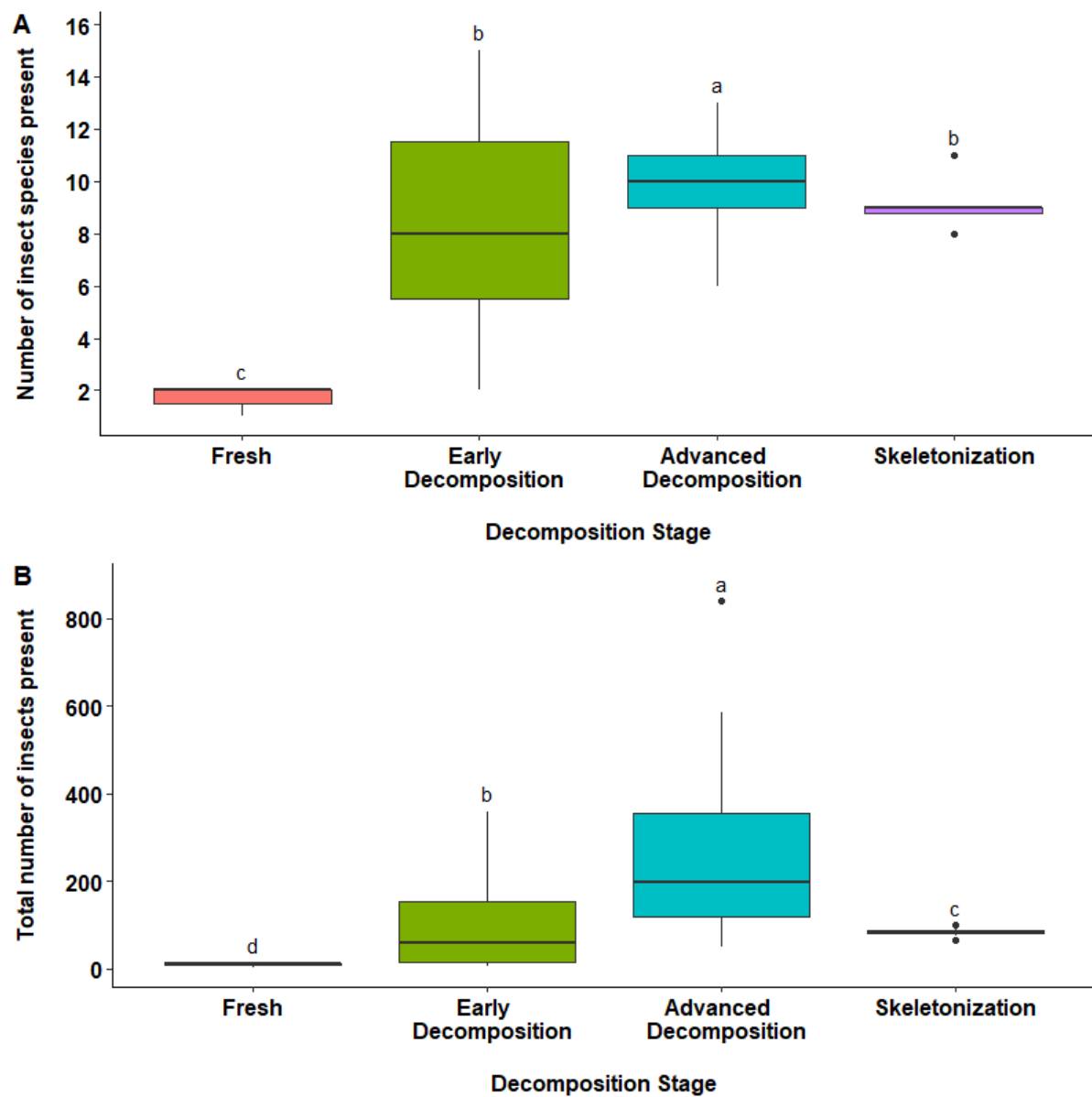


Figure 4.11: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the adult pig winter trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

4.2.5. Insect succession

Diptera succession

Within the family Calliphoridae, *Ca. croceipalpis* was the first colonizer, arriving as early as Day 1 while the pig carcass was still fresh (Figure 4.12). Following the transition of the carcass into the early decomposition stage on Day 6, *Ch. albiceps* was observed on the pig carcass on Day 8, while *Ch. chloropyga* and *Ca. vicina* arrived on Day 9 (Figure 4.12). A day after the arrival of *Ch. chloropyga* and *Ca. vicina* (i.e., Day 10), *Ch. megacephala* was observed interacting with the pig carcass. *Chrysomya marginalis* was observed to arrive on the pig carcass on Day 25, during the mid-phase of the early decomposition stage, while *L. cuprina* only appeared on the carcass on Day 86 after the carcass had already transitioned into the advanced decomposition stage.

Ca. croceipalpis persisted on the carcass until the advanced decomposition stage, while *Ch. megacephala* and *Ch. marginalis* maintained a fairly constant presence on the carcass from the early decomposition stage to the advanced decomposition stage. Both *Ch. albiceps* and *Ch. chloropyga* were present on the carcass from their time of arrival until the skeletonization stage. *Calliphora vicina* and *L. cuprina* were only present on the pig carcass during the early decomposition and advanced decomposition stages respectively. Of all the Calliphorid flies that were present on the carcass, only *Ch. chloropyga* oviposited on the pig carcass. On Day 16, first instar larvae of *Ch. chloropyga* were observed feeding on the flesh around the left ear and eye of the pig carcass. Furthermore, on Day 28, freshly laid egg masses of *Ch. chloropyga* were recorded on the right ear and abdominal area of the pig carcass. As decomposition progressed, the larvae of *Ch. chloropyga* continued to feed on the soft tissues of the carcass.

Adult members of the family Sarcophagidae were observed on the carcass on Day 9 during the early decomposition stage, and they were present albeit in limited numbers until the advanced decomposition stage. *Musca* sp. was present on the carcass as early as Day 2 while the carcass was fresh, and it maintained a constant presence until the late phase of the advanced decomposition stage (Figure 4.12). *Hydrotaea* sp. arrived on the pig carcass during the early decomposition stage specifically on Day 7, and further persisted until the skeletonization stage (Figure 4.12). *Suillia picta*, arrived on the carcass on Day 7, but could not persist on the carcass beyond the advanced decomposition stage (Figure 4.12).

Bibio sp. belonging to the family Bibionidae arrived during the mid-phase of the early decomposition stage and were present in the advanced decomposition and skeletonization

stages albeit sporadically. Members of the families Piophilidae and Phoridae both arrived on the carcass on Day 14 during the early decomposition stage, however, the Phorid flies maintained a limited presence until the advanced decomposition stage while the Piophilid flies were present until the skeletonization stage (Figure 4.12). Members of the families Drosophilidae, Anthomyiidae and Sepsidae arrived on the carcass during the mid-phase of early decomposition (i.e., between Days 24 and 28), but only the members of the family Anthomyiidae were recorded during the advanced decomposition stage of the pig carcass (Figure 4.12).

Coleoptera succession

Members of the families Scarabaeidae and Trogidae were the first colonizers, arriving as early as Day 10 during the early phase of the early decomposition stage (Figure 4.12). Members of the families Silphidae and Histeridae arrived on the pig carcass during the mid-phase of early decomposition on Days 24 and 25 respectively, while members of the family Staphylinidae arrived on Day 34 (Figure 4.12). Following the transition of the pig carcass into the advanced decomposition stage, members of the families Dermestidae and Cleridae were observed to arrive on the pig carcass on Days 56, and 86 respectively (Figure 4.12).

Among all other beetle families recorded on the pig carcass, only the members of the families Scarabaeidae and Trogidae were not observed on the carcass following its transition to the skeletonization stage. The larvae of Silphid beetles were recorded in the area underneath the carcass on Day 48, after the onset of the advanced decomposition stage (Figure 4.12). Towards the terminal phase of the advanced decomposition stage (i.e., Day 115), the larvae of Dermestid beetles were found on and underneath the carcass (Figure 4.12).

Hymenoptera and other insect orders succession

Crematogaster cf. *liengmei* was one of the first colonizers, arriving as early as Day 1 while the carcass was still fresh (Figure 4.12). On the same day, several individuals of *Crematogaster* cf. *liengmei* were seen actively feeding on the flesh behind the right ear of the pig (Figure 4.13A). On Day 2, several “dotted” bite marks/lesions were seen on the site of ant activity (Figure 4.13B). Between Days 2 and 8, changes in the coloration/appearance of the ant feeding site were noticeable (Figures 4.13C – E). As decomposition progressed, several *Crematogaster* cf. *liengmei* individuals were seen preying on and removing the eggs, larvae, and adult stages of blow flies in, on and around the pig carcass (see Supplementary Videos C2 – 4).

Alysia manducator was only observed to arrive on the pig carcass on Day 14 during the early decomposition stage (Figure 4.12). Following their arrival, several *A. manducator* individuals were observed in close association with the eggs and larvae of Calliphorid flies on the carcass. Furthermore, they were observed preying, stabbing, and dragging some larvae of Calliphorid flies on several occasions. *Alysia manducator* was able to persist on the carcass until the advanced decomposition stage while *Crematogaster* cf. *liengmei* maintained a constant presence until the skeletonization stage of the pig carcass (Figure 4.12). Members of other insect orders such as Blattodea were very limited in abundance and largely observed around the carcass across the early and advanced decomposition stages on Days 32, 34, and 77 only (Figure 4.12).



Figure 4.12: Daily abundance of adult insect species on the carcass in the adult pig winter trial.



Figure 4.12 (continued): Daily abundance of adult insect species on the carcass in the adult pig winter trial. Fresh stage: D0 – D5; Early decomposition: D6 – D47; Advanced decomposition: D48 – D121; Skeletonization: D122 – D149. L1 larvae and eggs of *Ch. chloropyga* were recorded on Days 16 and 28 respectively; Larvae of *Silphidae gen. sp.* and *Dermestidae gen. sp.* were respectively recorded on Days 48 and 115 until the end of the trial.

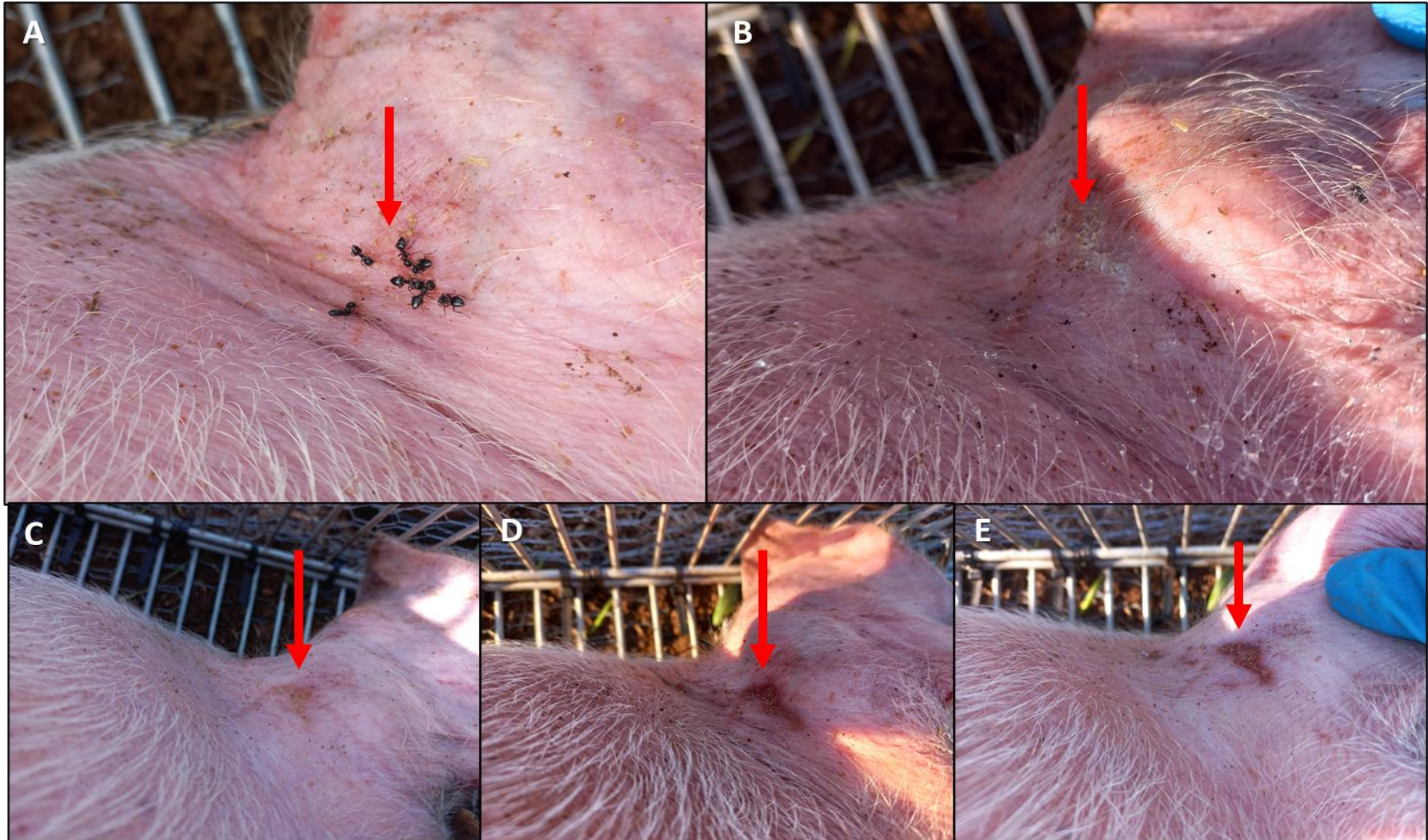


Figure 4.13: *Crematogaster* cf. *liengmei* individuals feeding on the flesh on the external part of the right ear on Day 1 (A), Changes in the coloration and appearance of the ant feeding site on Day 2 (B), Day 3 (C), Day 6 (D) and Day 8 (E) after adult pig carcass deployment in the winter season.

4.2.6. Influence of carcass size on insect species and individuals

As with the adult pig summer trial, the differences in ambient weather conditions such as temperature, rainfall and relative humidity between the adult pig winter trial and those of the neonate pig winter trials were analyzed before performing comparative analyses in the number of insect species and individuals.

Adult pig trial vs early winter neonate pig trial

There were significant differences in the ambient temperature ($p < 0.0001$) and relative humidity ($p < 0.001$) between the adult pig winter and early winter neonate pig trials. However, rainfall did not differ significantly ($p = 0.07$) between the two trials. The number of insect species differed significantly with respect to the size of the carcass (Likelihood Ratio $\chi^2 = 19.39$; $DF = 1$; $p < 0.0001$). The adult pig carcass attracted a significantly ($p < 0.05$) higher number of insect species when compared to the neonate pig carcasses (Figure 4.14A). Similarly, carcass size had a significant influence on the total number of insect individuals attracted to the pig carcasses (Likelihood Ratio $\chi^2 = 5939.8$; $DF = 1$; $p < 0.0001$). The adult pig carcass attracted a significantly ($p < 0.05$) higher number of insect individuals when compared to the neonate pig carcasses (Figure 4.14D).

Adult pig trial vs mid- winter neonate pig trial

Significant differences were found in the ambient temperature ($p < 0.0001$) between the adult pig winter and mid-winter neonate pig trials. However, ambient relative humidity ($p = 0.39$) and rainfall ($p = 0.82$) did not differ significantly between the two trials. Carcass size had a statistically significant influence on the number of insect species attracted to the pig carcasses (Likelihood Ratio $\chi^2 = 18.53$; $DF = 1$; $p < 0.0001$). The number of insect species attracted to the adult pig carcass was significantly ($p < 0.05$) higher than that of the neonate pig carcasses (Figure 4.14B). Likewise, carcass size had a significant effect on the total number of insect individuals attracted to the pig carcasses (Likelihood Ratio $\chi^2 = 5078.9$; $DF = 1$; $p < 0.0001$). The adult pig carcass attracted significantly ($p < 0.05$) higher numbers of insect individuals when compared to the neonate pig carcasses (Figure 4.14E).

Adult pig trial vs late winter neonate pig trial

There were significant differences in the ambient temperature ($p < 0.0001$), relative humidity ($p < 0.0001$), and rainfall ($p = 0.04$) the adult pig winter and late winter neonate pig trials. The size of the carcass had no significant impact on the number of insect species attracted to the

pig carcasses (Likelihood Ratio $\chi^2 = 0.34$; DF = 1; $p = 0.56$) (Figure 4.14C). Conversely, there was a significant difference in the total number of insect individuals with respect to size of the carcass (Likelihood Ratio $\chi^2 = 2950.3$; DF = 1; $p < 0.0001$). The adult pig carcass attracted significantly ($p < 0.05$) higher numbers of insect individuals when compared to the neonate pig carcasses (Figure 4.14F).

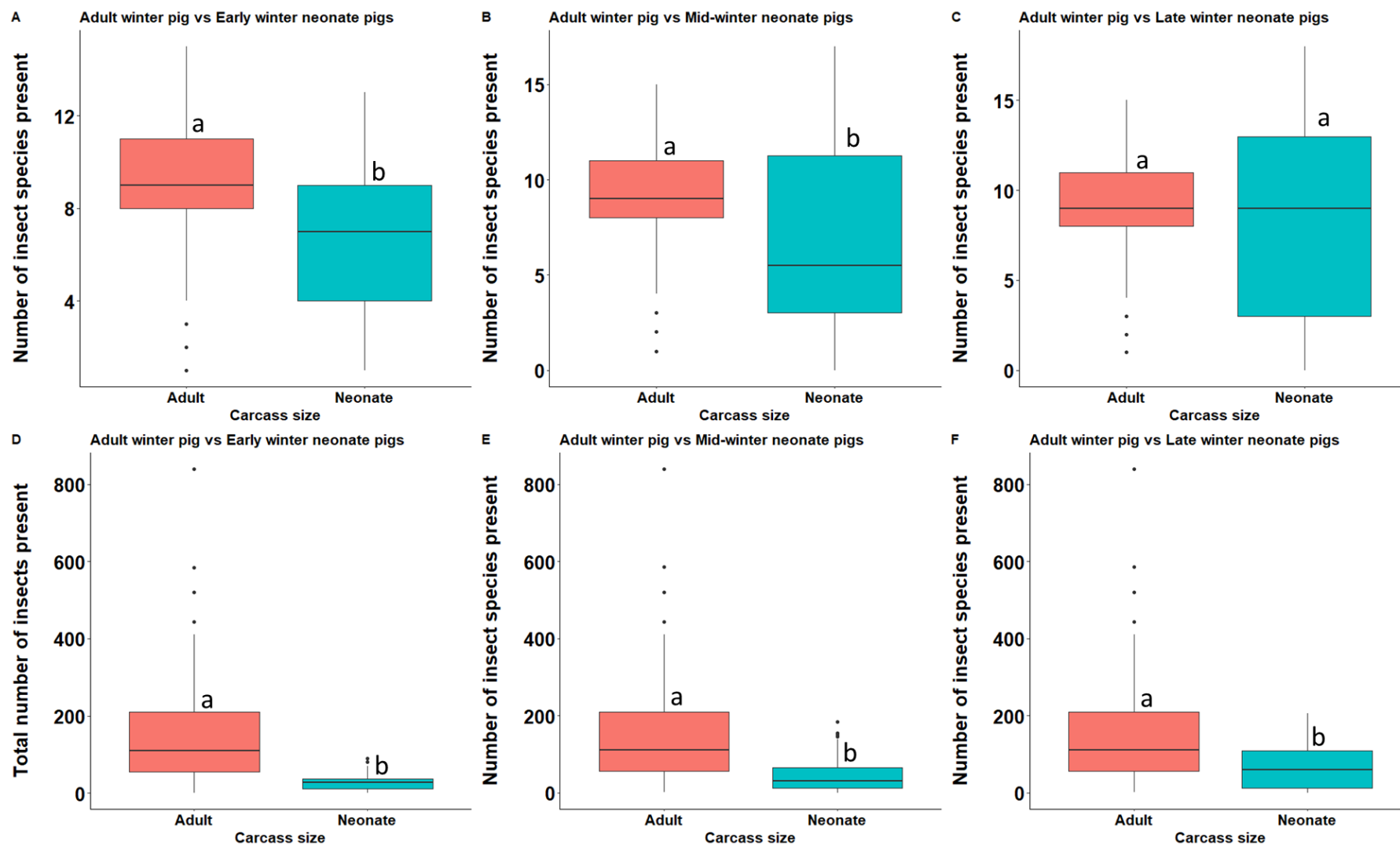


Figure 4.14: Box plots comparing the number of insect species (A – C) and total number of insects (D – E) attracted to the adult winter pig carcass and neonate pig early, mid-, and late winter carcasses. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

4.3. Discussion

In the Western Cape Province of South Africa, the summer and winter seasons are often the two seasons wherein more decomposing human remains are discovered as evidenced by recent retrospective case studies (Baliso *et al.*, 2019, 2023). Thus, to generate forensically relevant data that can typify observations recorded in actual forensic cases within the Western Cape Province, this study investigated the abundance, diversity, and successional patterns of insect species associated with singly deployed adult pig carcasses in the summer and winter seasons respectively, within the Table Mountain region. Furthermore, detailed analysis and comparison of the insect assemblage and their successional patterns generated from this study with those earlier generated from the neonate pig carcasses across the four seasons were carried out.

Insect diversity and abundance across various decomposition stages

In agreement with the observations recorded during the neonate pig summer and winter seasonal trials, the advanced decomposition and skeletonization stages attracted the highest number of insect species and individuals in the singly deployed adult pig summer and winter trials.

Insect species assemblage and succession across the summer and winter seasons and comparisons with the neonate pig insect assemblage and succession data

Diptera

Chrysomya megacephala was observed to be one of the first insect colonizers of the adult pig carcasses across the summer and winter seasons. The early presence of *Ch. megacephala* on the adult pig carcass in the summer season is in agreement with the findings of the neonate pig trials across the four seasons, and other similar studies on large-size pig carcasses (weights $\geq$ 13kg) in the summer season (Sebastião and Prado e Castro, 2019; Hu *et al.*, 2023). In contrast, other similar studies utilizing adult-sized pig carcasses within South Africa did not report the presence of this blow fly species (Kelly, 2006; Kolver, 2009; Gilbert, 2014; Botham, 2016; Finaughty, 2019; Tembe and Mukatirawa, 2021, Tembe *et al.*, 2021a, b).

In the neonate pig summer trials, the immature stages of *Ch. megacephala* were recorded on the carcasses. However, in this summer trial with an adult pig carcass, the immature stages of *Ch. megacephala* were not observed. Furthermore, in this summer seasonal trial, *Ch. megacephala* was not the most abundant Calliphorid fly present on the pig carcass. Yet, *Ch. megacephala* dominated the Dipteran community in the neonate pig summer trials.

The fact that the eggs/larvae of *Ch. megacephala* were not recorded on the adult pig carcass (deployed in the early summer period) agrees with the findings of the neonate pig early summer seasonal trial. Similarly, in the adult pig winter trial, the abundance of *Ch. megacephala* was low and their eggs were not recorded on the pig carcass. The low abundance of *Ch. megacephala* and the complete absence of their eggs/larvae on the adult pig carcass in the winter season was not an unusual observation and this agrees with the findings of the neonate pig winter seasonal trials.

Calliphora croceipalpis and *Ca. vicina* were one of the forensically important blow flies collected during the neonate pig spring, summer, and winter seasonal trials. The prevalence of these two blow fly species in the winter season of South Africa is not unusual (Kelly, 2006; Gilbert, 2014). Yet, Finaughty (2019) did not record *Ca. croceipalpis* during the summer and winter seasons of his field decomposition studies utilizing adult pig carcasses. This observation further supports the hypothesis that insect species assemblage could differ between localities even within the same region. In addition, it supports the hypothesis that different weather conditions attract different species and there may be seasonal differences between years.

Contrary to the observations recorded in the neonate pig winter trials, the immature stages of *Ca. croceipalpis* and *Ca. vicina* were not recorded on the adult pig carcass used in this winter seasonal trial. For *Ca. croceipalpis*, this may be attributed to its preference to breed on small-sized carcasses (Meskin, 1986; Richards *et al.*, 2009b) while for *Ca. vicina*, this may be attributed to their low abundance which can be linked to their invasive status and limited distribution within the Western Cape Province (Williams and Villet, 2006b). Additionally, it is possible that the adult female *Ca. croceipalpis* and *Ca. vicina* individuals interacting with the pig carcass in the winter season were not gravid and thus not having any eggs to lay on the carcass.

Chrysomya chloropyga and *Ch. albiceps* were both one of the early colonizers of the adult pig carcass in the summer season, and their immature stages were recorded on the carcass. However, in the adult pig winter season trial, only the immature stages of *Ch. chloropyga* were recorded on the carcass. The abundance and dominance of *Ch. chloropyga* in the summer and winter seasons as seen here is not uncommon and this echoes the findings of the neonate pig summer and winter trials, and those of other similar studies in South Africa (Kelly *et al.*, 2011). Similarly, the abundance of *Ch. albiceps* in the summer season as seen

here is in concordance with the observations recorded in the neonate pig summer trials and those of other similar studies on large-sized carcasses conducted in the summer season (Kelly *et al.*, 2009, 2011; Gibert, 2016; Finaughty, 2019).

Interestingly, within the same region, Finaughty (2019) noted the entire absence of *Ch. chloropyga* on decomposing adult pig carcasses in the summer season and an increase in the abundance of this same blow fly species following the transition into the late winter and early spring seasonal period. The fact that the immature stages of *Ch. chloropyga* and *Ch. albiceps* were recorded on the adult pig carcass in the summer season was not surprising as they were earlier found on the neonate pig carcasses in the summer season. In this study, the high abundance and dominance of *Ch. chloropyga* in the adult pig summer and winter seasonal trials, and its successful oviposition on the pig carcasses may be attributed to its thermal and seasonal flexibility as earlier mentioned (Richards *et al.*, 2009a, b).

In the literature it has been suggested that *Ch. marginalis* prefers large-sized carcasses (Braack, 1984; Meskin, 1986; Sebastião and Prado e Castro, 2019). Despite the preference of *Ch. marginalis* for the summer season (Braack, 1986; Kelly *et al.*, 2011), the abundance of *Ch. marginalis* in the adult pig summer trial was very low in comparison to its congeners, agreeing with the summer seasonal findings of Finaughty (2019). Furthermore, in relation to carcass size, the abundance of *Ch. marginalis* on the adult pig carcass was not considerably different from that recorded on the neonate pigs during the same season. In fact, the immature stages of *Ch. marginalis* were not recorded on the adult pig carcass, which was not the case in the neonate pig summer trials where they recorded on one of the carcasses. Based on these preliminary observations, there appears to be no preference for large-sized carcasses by *Ch. marginalis*, at least in the habitat under study.

As mentioned earlier in Chapter 3, *H. fernandica* has been speculated to exhibit a preference for small-sized carcasses (Meskin, 1986; Ekanem and Dike, 2010; De Jong and Krell, 2011). However, in the adult pig summer trial, the abundance and persistence of *H. fernandica* on the pig carcass was not substantially different from what was recorded during the neonate pig summer trials. The immature stages of *H. fernandica* were recorded on one of the neonate pig carcasses in the summer season, however, this was not the case in the adult pig summer trial. In contrast to the findings of the neonate pig winter seasonal trials, *H. fernandica* was not recorded in the adult pig winter seasonal trial. The abundance of other Calliphorid flies

such as *L. cuprina*, and *L. sericata* in this summer trial was very low and is congruent with the observations recorded in the summer and winter neonate pig trials.

In the adult pig summer and winter seasonal trials, the abundance of the members of the family Sarcophagidae was generally low when compared to the Calliphoridae and Muscidae flies, and this was consistent with the findings of the neonate pig trials across the summer and winter seasons. Despite their presence on the adult pig carcasses, none of the Sarcophagid adult flies deposited their larvae on the pig carcasses. This agrees with the findings of Finaughty (2019) where no Sarcophagid species deposited their larvae on the adult pig carcasses used in his summer and winter seasonal studies. However, it significantly contradicts what was recorded during the neonate pig summer and winter seasonal periods wherein the larvae of Sarcophagid flies were recorded on carcasses. The colonization patterns of other carrion-associated flies such as *Musca* sp., *Hydrotaea* sp., *S. picta*, and members of the families Drosophilidae, Phoridae, Sepsidae, Piophilidae, and Anthomyiidae in the adult pig summer and winter trials were generally similar to what was observed in the neonate pig summer and winter trials and will not be discussed any further.

Hymenoptera

In agreement with the observations recorded in the neonate pig summer and winter seasonal trials, several individuals of *Crematogaster* cf. *liengmei* were noticed on the adult pig carcasses in the summer and winter seasons as early as Day 1. In the adult pig summer seasonal trial, several individuals of *Crematogaster* cf. *liengmei* were observed on the pig carcass feeding and creating tiny bite marks on the flesh between the hindlimbs and external part of the left ear. Similarly, in the adult pig winter trial, they created several “dotted” bite-marks/lesions on the flesh of the pig’s right ear, all of which began to change in appearance and coloration with increased exposure duration. Furthermore, they were observed on different occasions, preying on, and removing the eggs, larvae, and adult stages of blow flies in, on and around the adult pig carcasses, and this was more pronounced in the winter season.

The necrophagous and predatory behaviors of *Crematogaster* cf. *liengmei* on the adult pig carcasses were similar to what was observed in the neonate pig spring, autumn and winter seasonal trials (Adetimehin *et al.*, 2022). Thus, it can be concluded that ants can exert similar predatory and necrophagic behaviors on neonate and adult-sized vertebrate remains. It is worthy to note that, in the neonate pig summer trials, the infliction of tiny bite marks on the pig carcass was not observed. This variation in ant feeding behavior on the adult and neonate

pig carcasses in the summer seasonal period may be attributed to the proximity of the ant nests to the carcasses, availability of foraging individuals and the nests' nutritional status (Adetimehin *et al.*, 2022). Furthermore, within the vicinity of the adult pig carcasses' deployment sites, some ant nests were observed at approximately 1.5m to the experimental setups. This may have contributed to their prompt location and consumption of the flesh of the adult pig carcasses shortly after their arrival.

The presence, and behavior of the parasitic wasp *A. manducator* in the adult pig summer trial and that of the neonate pig summer trial was similar. Therein, no adult wasps were observed parasitizing or preying on the eggs or larvae of blow flies on the adult pig carcass. The vigorous feeding activities of the larvae all over the carcass, alongside their rapid development mediated by the high ambient temperature may have contributed to the inability of the wasps to prey or parasitize the blow fly larvae feeding on the carcass in the summer season (Adetimehin *et al.*, 2023a). In the adult pig winter seasonal trial, several *A. manducator* species were observed in close association with the eggs and larvae of Calliphorid flies on the carcass. This was the case in the neonate pig winter trials. Furthermore, they were seen preying, stabbing, and dragging some larvae of Calliphorid flies on several occasions. The ability of parasitic wasps in detecting vertebrate carrion and carrion-associated insects have been documented (see Adetimehin *et al.*, 2023a). Furthermore, the ability of parasitoid wasps including *A. manducator* to prey on, stab and drag their host insects and the forensic implications of these behaviors have been detailed in Adetimehin *et al.* (2023a) with videographic evidence.

Coleoptera

As decomposition progressed into the late phase of early decomposition and advanced decomposition, members of the families Silphidae, Histeridae, Staphylinidae Dermestidae and Cleridae began to colonize the adult pig carcasses in the summer and winter seasons. This is similar to what was observed in the neonate pig summer and winter trials. However, a substantial difference in the beetle community on the adult pig carcasses in the summer and winter seasons when compared to those of the neonate pig carcasses within the same seasons was the considerable increase in the number of Clerid beetles present on the adult pig carcasses. For instance, very few Clerid beetles were collected during the neonate pig summer trials, while in the winter trials, Clerid beetles were entirely absent.

In the summer and winter seasons of South Africa, studies utilizing large-sized pig carcasses in establishing baseline entomological data have reported considerably high numbers of Clerid beetles being attracted to the carrion (Kelly, 2006; Finaughty, 2019). This was the case in the adult pig trials, but not the neonate pig trials. For the neonate pig trials, this is not surprising as the underrepresentation of selected beetle families including the family Cleridae on smaller-sized vertebrate remains has been reported (Matuszewski *et al.*, 2016). Of all the beetles that were attracted to the adult pig carcass in the summer season, only the larvae of Dermestid beetles were observed on the carcass. This observation is in agreement with the summer findings of Finaughty (2019). Yet, it contradicts the observations in the neonate pig summer trials where no beetle larvae were observed on the pig carcasses. In the winter season, the larvae of Silphid and Dermestid beetles were recorded on the pig carcass, and this is in agreement with the findings of the neonate pig trials (for the Silphid beetles) and those of Finaughty (2019) in the winter season.

Incidental observation: unidirectional migration of blow flow together with the decomposition fluids and forensic implication

An interesting observation that was not expected during this trial was the synchronized and unidirectional mass migration of blow fly larvae on Day 5 of the trial. The blow fly larvae were seen migrating downhill in a southwesterly direction together with the decomposition fluids oozing out from the carcass. The “larval migration stream” primarily consisted of the larvae of *Ch. albiceps* and *Ch. chloropyga*. The observed synchronized and unidirectional migration of blow fly larvae within and in the direction of the flow of the decomposition fluids is unusual as such phenomenon was not observed in all the previous experimental trials on neonate and adult pig carcasses in different seasons within the same study location. Furthermore, such mass migration of blow fly larvae together with the decomposition fluids was not observed in the summer decomposition and insect successional study of Finaughty (2019). In accordance with Goddard *et al.* (2020), it is worthy to note the possibility of missing out on the timing of the mass dispersal of blow fly larvae during the previous decomposition and insect successional studies within the same study location by virtue of the field sampling time, which was at most ~2 hours for every sampling day.

The characteristic downhill mass migration of blow fly larvae together with the decomposition fluids in the southwestern direction may be linked to the topography of the pig deployment site and associated gravitational influence (Gomes *et al.*, 2006; Lashley *et al.*,

2018). The downhill dispersal of blow fly larvae was also documented in the study by Lashley *et al.* (2018). On the 5th day after the deployment of a 725kg of carrion biomass, the authors observed millions of blow fly larvae from the study location dispersing *en masse* in a synchronized manner and in a unified direction. Secondly, it is speculated that the coordinated dispersal of blow fly larvae within and in the direction of flow of the decomposition fluids may be one of the mechanisms employed by blow fly larvae to reduce the rate of desiccation and to enable them travel easily and further away from the food source during their post-feeding migratory period (Heinrich, 2013; Goddard *et al.*, 2020). Echoing the suggestion by Heinrich (2013), the reason for the unidirectional, *en masse* and synchronized dispersal of blow fly larvae from the food source (i.e., vertebrate carrion) is unclear and further studies are needed to disentangle what triggers this unique larval migratory pattern.

The downhill mass migratory pattern of blow fly larvae with the decomposition fluids from a vertebrate carrion can increase the area of the cadaver decomposition island which in turn can potentially complicate the location/discovery of clandestine cadaver deposition sites especially when human cadaver detection dogs are utilized (Aitkenhead-Peterson *et al.*, 2012; Lashley *et al.*, 2018; Glavaš and Pintar, 2019). Although, the experimental design of this study excluded vertebrate scavengers from interacting with the pig carcass, the south-westerly directional flow of the decomposition fluids from the carcass might be misconstrued as scavenger induced dispersal of the carrion appendages (Steadman *et al.*, 2018; Barton *et al.*, 2020). In addition, this can be misinterpreted as the dragging trail of a body left behind by assailant(s) as part of the cadaver concealment process.

Understanding the timing and pattern of dispersal of post-feeding fly larvae in each locality and geographical region is crucial especially if the oldest immature insect specimens found in/on/around a corpse are to be employed in the estimation of the PMImin (Buchan and Anderson, 2001; Amendt *et al.*, 2011). This is because the PMImin can be miscalculated if older immature insect specimens dispersing from the corpse are not considered and collected (Lewis and Benbow, 2011). While further studies are needed to understand the possible ecological factors triggering this unique behavior in blow fly larvae, and the subsequent impact in and around the surrounding environments, the unexpected observations on the mass dispersal of blow fly larvae together with the decomposition fluids adds to the existing but scanty literature on this phenomenon and further supports previous speculations in similarly cited studies.

Influence of carcass size on insect species richness and abundance

Finally, based on the results obtained when comparing the number of insect species and individuals attracted to the adult and neonate pig carcasses across the summer and winter seasonal periods, it seems fair to suggest that carcass size does exert some degree of influence on the number of insect species and individuals attracted to the carcasses. In the winter seasonal period, the influence of carcass size on the species richness and abundance of carrion-associated insects attracted to a cadaver was more pronounced. The significantly higher number of insect species and individuals attracted to the adult pig carcass within the winter seasonal period may be attributed to the body mass of the carcass, and the extended decomposition period of the carcass.

In the literature, the role that carcass size plays on insect diversity and abundance is controversial (see Kuusela and Hanski, 1982; Hewadikaram and Goff, 1991; Azwandi *et al.*, 2013; Mashaly *et al.*, 2019a). For this reason, it is crucial to note that the results generated from the outright comparisons as performed in this study should be used with caution as the trials were not conducted simultaneously. While the comparisons here are preliminary, future studies within the region should simultaneously deploy several carcasses with different body masses ranging from small (2 – 5kg), to medium (15 – 30kg), and large (> 50kg) to examine how the size of the carcass influences the diversity and abundance of carrion-associated insects in the summer and winter seasonal periods.

Conclusion

This study has revealed that the insect species that deposited their eggs on the neonate pig carcasses were generally similar to those that oviposited on the adult pig carcasses across the summer and winter seasons. Furthermore, this study revealed that ants can exert similar predatory and necrophagic behaviors, as documented in the neonate pig decomposition trials, on adult-sized pig carcasses. Lastly, carcass size was observed to exert significant influences on the insect assemblages, and this suggests the need for further detailed studies on the role of carcass size on carrion-associated insect assemblages across several seasons.

Chapter 5: Retrospective Insect Data from Human Forensic Cases

5.1. Case evaluation

During the six-year period, between 1 January 2015 and 31 December 2022, a total of 14 entomological reports were produced. Eleven cases (78.6%) consisted of human remains found decomposing in outdoor environments while the remaining three cases (21.4%) comprised of human remains found decomposing indoors. Most of the cases in which the entomological reports were written were from the Paarl mortuary (n = 5; 36%) followed by the Salt River (n = 4; 29%), Tygerberg (n = 3; 21%), Malmesbury (n = 1; 7%) and Vredendal (n = 1; 7%) mortuaries (Figure 5.1).

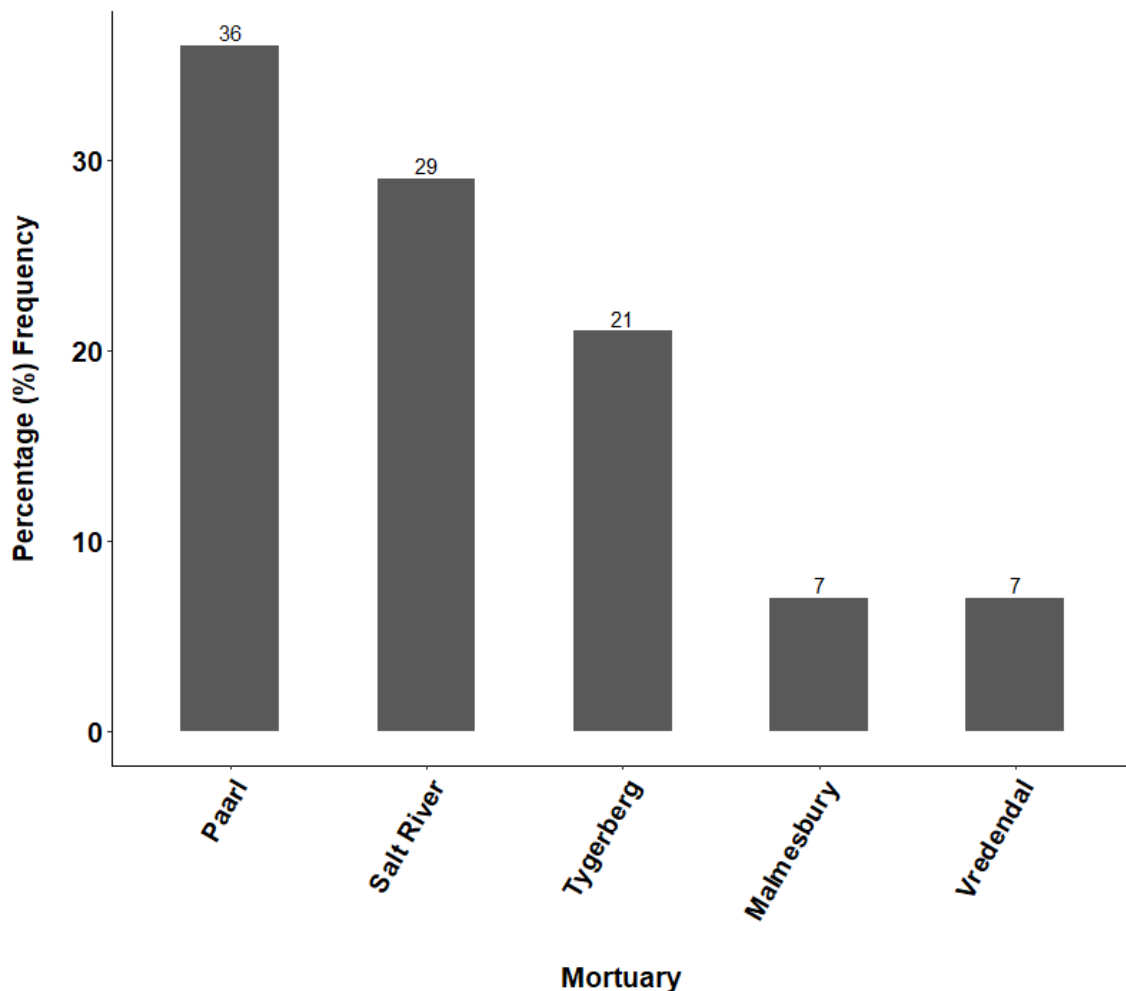


Figure 5.1: Percentage frequency of the cases assessed by CapeFORTE in relation to the mortuary location.

With respect to the sex of the decedents in the entomological reports analyzed, males accounted for 57.1% (n = 8) while females accounted for 42.9% (n = 6) (Table D1). The level of decomposition of the human remains observed in these cases were predominantly cases with signs of early decomposition (n = 8; 57.1%) followed by cases which displayed evidence of advanced decomposition (n = 4; 28.6%). Only two cases (n = 14.3%) were in the skeletonization stage (Figure 5.2).

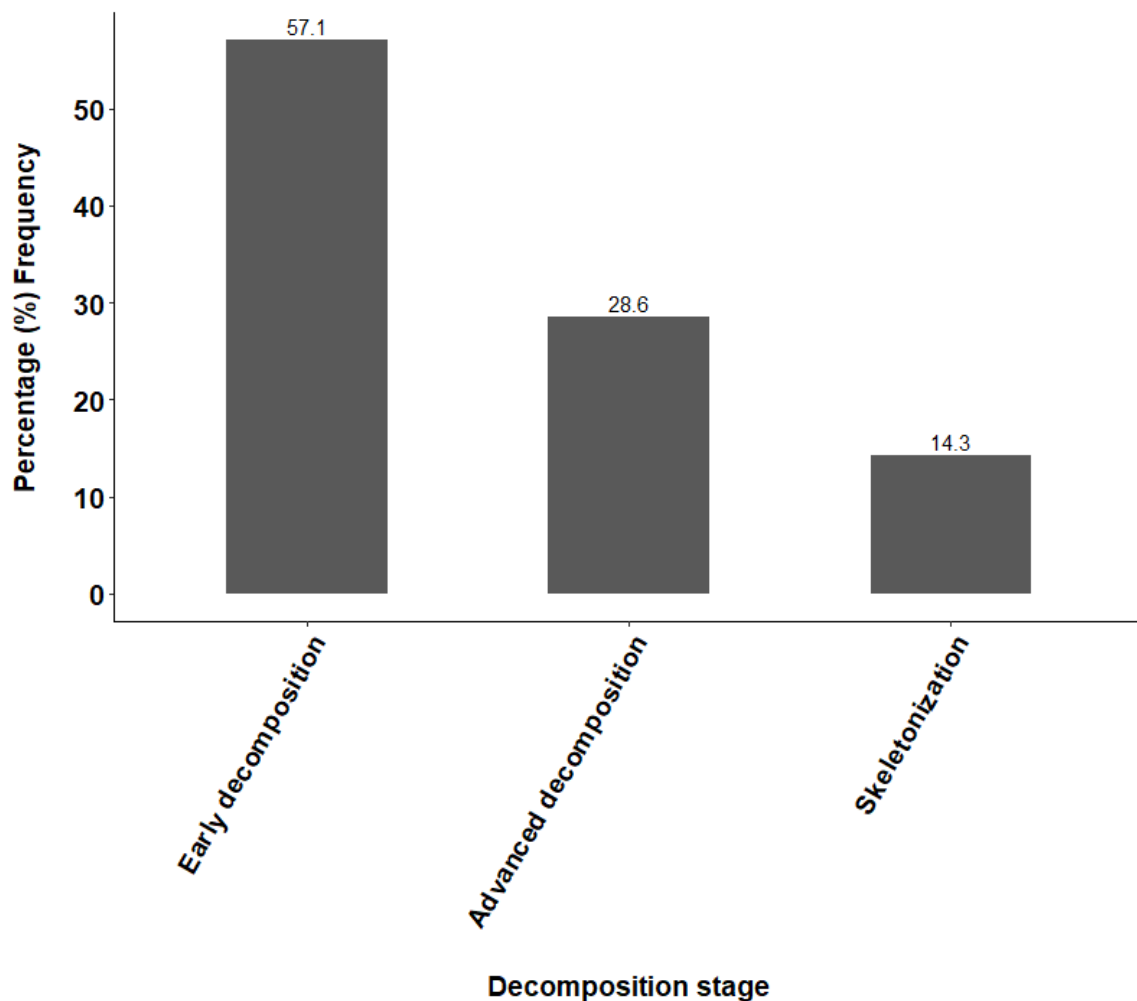


Figure 5.2: Percentage frequency of the cases assessed by CapeFORTE in relation to the decomposition stage of the human remains.

5.2. Temporality

Temporally, most entomological cases ($n = 7$; 50%) occurred within the summer seasonal period (Figure 5.3). Three cases (21.4%) occurred within the spring season, while 2 cases (14.3%) each occurred in the autumn and winter seasons respectively (Figure 5.3).

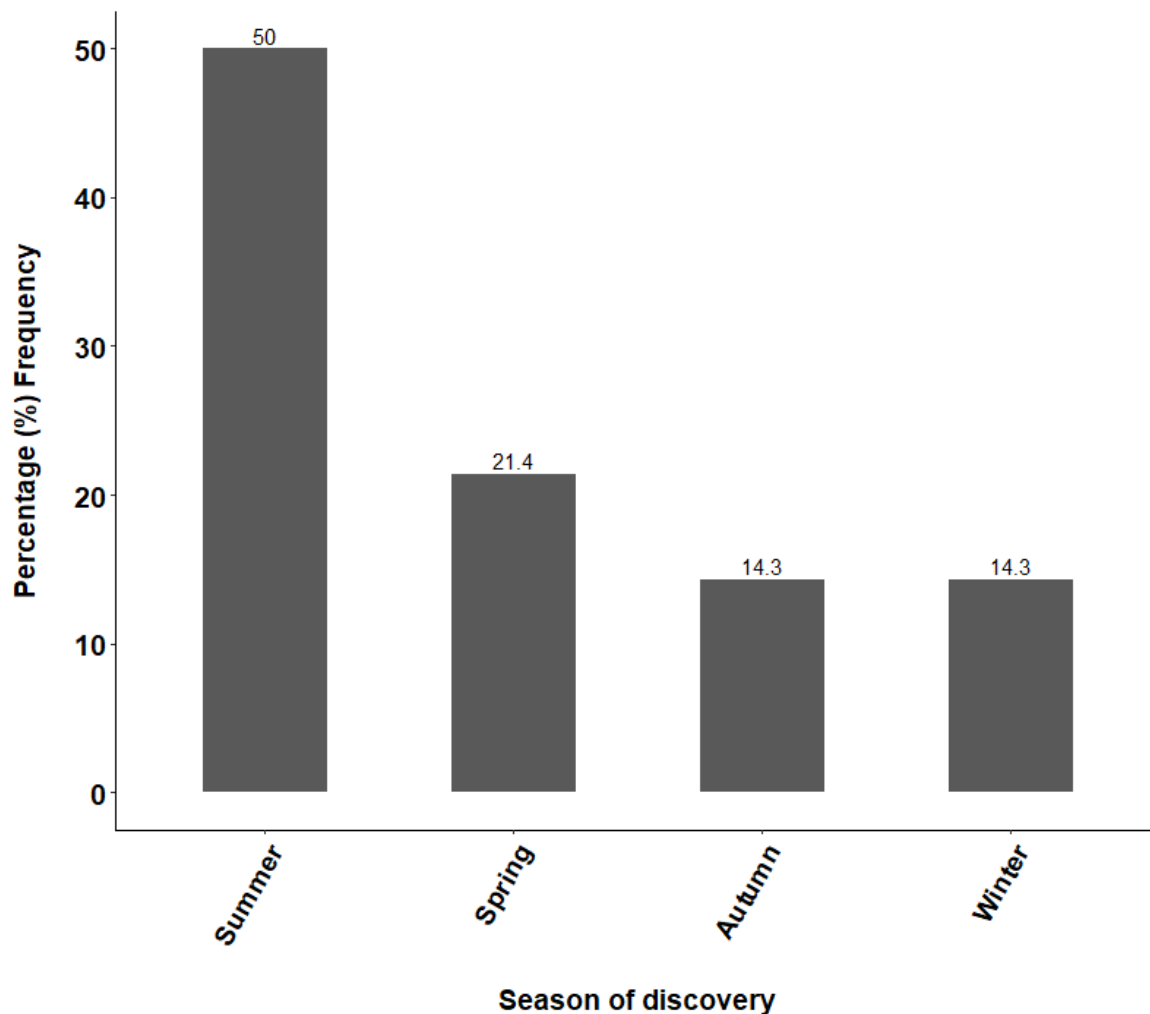


Figure 5.3: Percentage frequency of the cases assessed by CapeFORTE in relation to the season of discovery.

The month wherein the highest number of human remains (3; 21.4%) were discovered was January (Figure 5.4). This was followed by February (2; 14.3%), August (2; 14.3%), October (2; 14.3%), December (2; 14.3%), March (1; 7.1%), May (1; 7.1%), and November (1; 7.1%). With respect to the year, a total of 6 cases (42.9%) occurred in 2016, and this was followed by the year 2022 wherein a total of three cases (21.4%) occurred. Two cases (14.3%) occurred in

2017, and this was followed by the years 2015 (n = 1; 7.1%), 2018 (n = 1; 7.1%), and 2021 (n = 1; 7.1%).

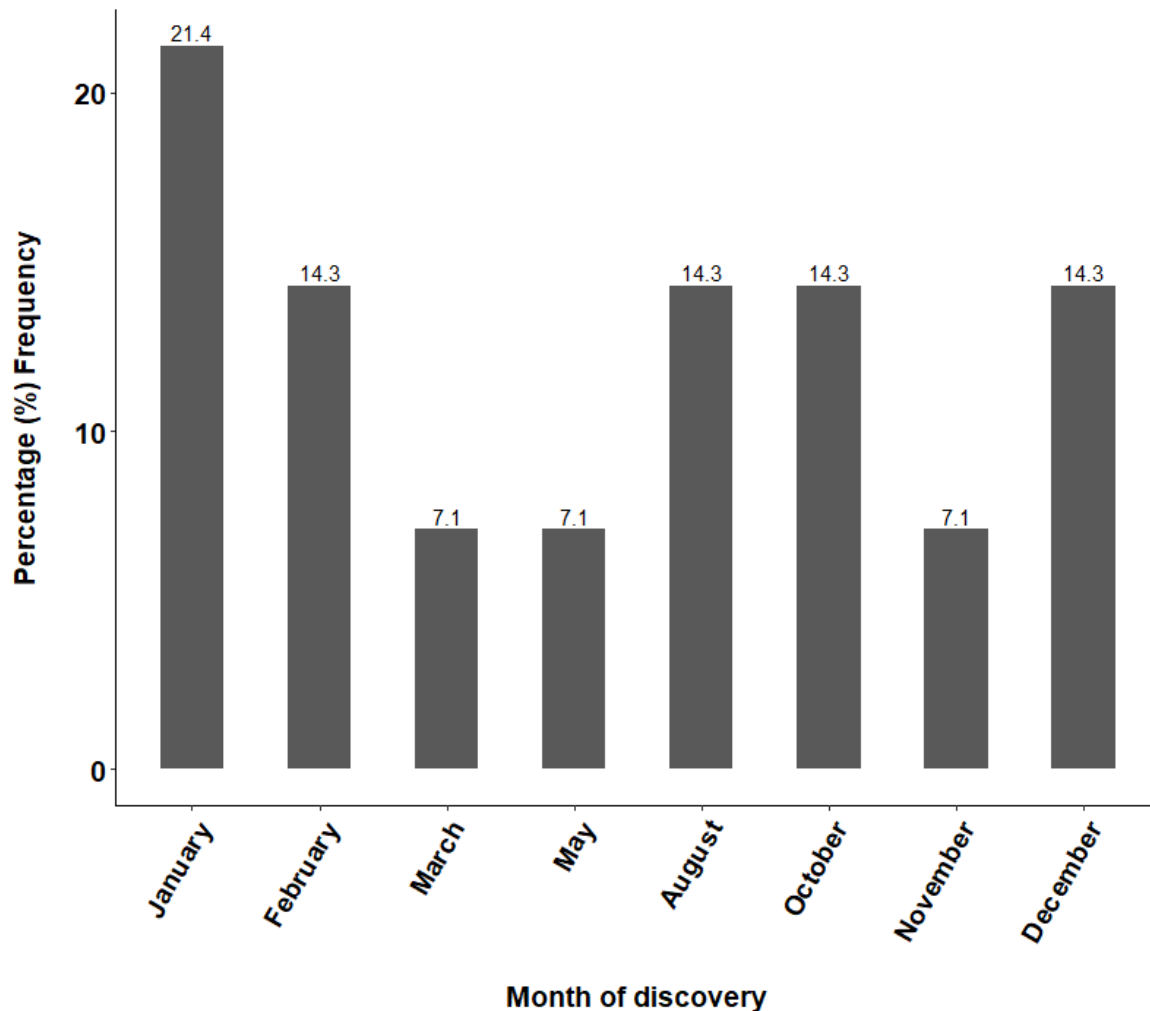


Figure 5.4: Percentage frequency of the cases assessed by CapeFORTE in relation to the month of discovery.

5.3. Insect colonization and identity

Within the six-year period, the entomological reports associated with these cases recorded a total of 23 insect species comprising 2 insect orders and 10 insect families. These insect orders were Diptera and Coleoptera, while the insect families were Calliphoridae, Sarcophagidae, Muscidae, Piophilidae, Sepsidae, Dermestidae, Cleridae, Histeridae, Staphylinidae and Silphidae (Table 5.1).

5.3.1. Entomological specimen sampling scene

In most of the cases, the entomological specimens were sampled during autopsy ($n = 11$; 78.6%). In two cases ($n = 2$; 14.3%), the entomological specimens were sampled at the death scene, while in only one case ($n = 1$; 7.1%), the entomological specimens were sampled during autopsy and at the scene of death. In half of the total cases ($n = 7$; 50%), the entomological specimens were collected by the attendant forensic pathologists, while in the other half, a member of CapeFORTE collected the entomological specimens.

5.3.2. Insect colonization rate

In only one case ($n = 1$, 7%) were the human remains colonized by a single insect species. Two cases ($n = 2$, 14%) had the human remains colonized by six insect species while three cases ($n = 3$, 21%) were colonized by five insect species. In most cases ($n = 4$, 29%), the human remains were colonized by two and four insect species (Figure 5.5, Table D1).

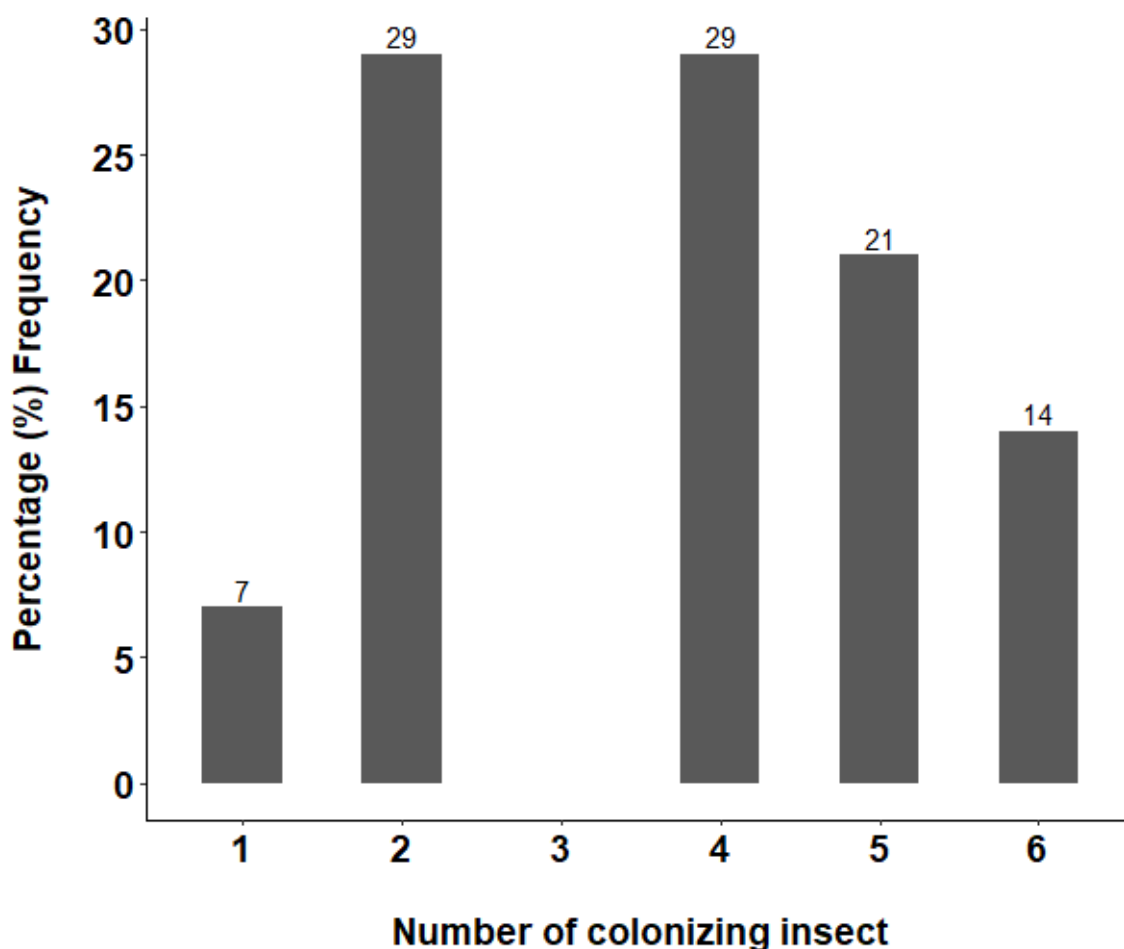


Figure 5.5: Percentage frequency of the cases assessed by CapeFORTE in relation to the number of colonizing insect species.

Within the order Diptera, members of the families Calliphoridae (100%, 14 out of 14 cases), and Muscidae (21.4%, 3 out of 14 cases) were the two most frequently occurring families found colonizing the human remains (Table 5.1). With respect to case frequency, *Ch. albiceps* (71.4%, 10 out of 14 cases) and *Ch. marginalis* (50%, 7 out of 14 cases) were the most common fly species collected from the human remains (Table 5.1, Tables D1 & D2). In the order Coleoptera, members of the family Cleridae (28.6%, 4 out of 14 cases) were the most frequently occurring beetle family on the human remains, followed by the families Dermestidae (21.4%, 3 out of 14 cases) and Histeridae (21.4%, 3 out of 14 cases) (Table 5.1, Tables D1 & D2).

5.3.3. Scene of discovery

All the outdoor decomposing human remains were colonized by members of the family Calliphoridae (100%, 11 out of 11 cases), followed by the families Muscidae (18.2%, 2 out of 11 cases) and Piophilidae (18.2%, 2 out of 11 cases) (Table 5.1). In the indoor cases, all the human remains were colonized by members of the family Calliphoridae (100%, 3 out of 3 cases). Members of the families Muscidae and Sarcophagidae respectively colonized 33.3% (1 out of 3 cases for each family) of the indoor decomposing human remains. None of the members of the beetle families mentioned above were found on the indoor decomposing human remains. The most common Calliphorid flies collected from outdoor decomposing human remains were *Ch. albiceps* (72.7%, 8 out of 11 cases), followed by *Ch. marginalis* (54.5%, 6 out of 11 cases) and *Ch. chloropyga* (45.5%, 5 out of 11 cases). In the indoor cases, both *Ch. albiceps* and *L. cuprina* had the same frequencies (66.7%, 2 out of the 3 cases for each species). Members of the family Sarcophagidae were equal in occurrence in both indoor and outdoor decomposing human remains (50%, 1 out of 2 for each scene). The most common beetle species collected from the outdoor cases was Cleridae gen. sp. (36.4%, 4 out of 11 cases).

Table 5.1: Case frequency of insect species collected from 14 human forensic cases (n = number of cases) in indoor/ outdoor scenes within the Western Cape Province of South Africa from 2015 to 2022.

Total (n = 14)				
Family	Species	Indoor (n = 3; 21.4%)	Outdoor (n = 11; 78.6%)	Total (% in 14 cases)
Calliphoridae		100%	100%	100%
	<i>Ch. chloropyga</i>	0	5 (45.5%)	35.7%
	<i>Ch. albiceps</i>	2 (66.7%)	8 (72.7%)	71.4%
	<i>Ch. megacephala</i>	0	1 (9.1%)	7.1%
	<i>Ch. marginalis</i>	1 (33.3%)	6 (54.5%)	50%
	<i>Ca. vicina</i>	1 (33.3%)	0	7.1%
	<i>L. sericata</i>	1 (33.3%)	1 (9.1%)	14.3%
	<i>L. cuprina</i>	2 (66.7%)	0	14.3%
	<i>Lucilia</i> spp.	0	1 (9.1%)	7.1%
	<i>H. fernanidca</i>	1 (33.3%)	0	7.1%
	Calliphoridae gen. sp.	0	1 (9.1%)	7.1%
Sarcophagidae		33.3%	9.1%	14.3%
	Sarcophagidae gen. sp.	1 (33.3%)	1 (9.1%)	14.3%
Muscidae		33.3%	18.2%	21.4%
	<i>M. domestica</i>	0	1 (9.1%)	7.1%
	<i>M. prolapsa</i>	1 (33.3%)	0	7.1%
	<i>H. capensis</i>	0	1 (9.1%)	7.1%
Piophilidae			18.2%	14.3%
	Piophilidae gen. sp.	0	2 (18.2%)	14.3%
Sepsidae			9.1%	7.1%
	Sepsidae gen. sp.	0	1 (9.1%)	7.1%
Dermestidae			27.3%	21.4%
	<i>D. maculatus</i> + Dermestidae gen. sp.	0	3 (27.3%)	21.4%
Cleridae			36.4%	28.6%
	<i>N. rufipes</i> + Cleridae gen. sp.	0	4 (36.4%)	28.6%
Histeridae			27.3%	21.4%
	Histeridae gen. sp.	0	3 (27.3%)	21.4%
Staphylinidae			9.1%	7.1%
	Staphylinidae gen. sp.	0	1 (9.1%)	7.1%
Silphidae			18.2%	14.3%
	<i>T. mutilatus</i>	0	2 (18.2%)	14.3%

5.3.4. Decomposition stage

Within the order Diptera, *Ch. albiceps* (75%, 6 out of the 8 cases) was the most common fly collected from human remains in the early decomposition stage (Table 5.2). *Chrysomya marginalis* (75%, 3 out of the 4 cases) and *Ch. albiceps* (75%, 3 out of the 4 cases) were the most frequently collected flies from human remains in the advanced decomposition stage. Only 50% (1 out of 2 cases) of the human remains in the skeletonization stage was infested by *Ch. chloropyga*, *Ch. albiceps* and *Ch. marginalis*. Within the order Coleoptera, members of the family Histeridae (25%, 2 out of the 8 cases) were the most common beetles collected from human remains in the early decomposition stage. Members of the family Cleridae colonized 100% (4 out of 4 cases) of the human remains in the advanced decomposition stage. Members of the family Staphylinidae were collected from 50% (1 out of the 2 cases) of the discovered human remains in the skeletonization stage.

Table 5.2: Case frequency of insect species collected from 14 human forensic cases (n = number of cases) in different stages of decomposition within the Western Cape Province of South Africa from 2015 to 2022.

Stage of decomposition (n = 14)				
Family	Species	Early decomposition (n = 8; 57.1%)	Advanced decomposition (n = 4; 28.6%)	Skeletonization (n = 2; 14.3%)
Calliphoridae				
	<i>Ch. chloropyga</i>	3 (37.5%)	1 (25%)	1 (50%)
	<i>Ch. albiceps</i>	6 (75%)	3 (75%)	1 (50%)
	<i>Ch. megacephala</i>	0	1 (25%)	0
	<i>Ch. marginalis</i>	3 (37.5%)	3 (75%)	1 (50%)
	<i>Ca. vicina</i>	1 (12.5%)	0	0
	<i>L. sericata</i>	2 (25%)	0	0
	<i>L. cuprina</i>	2 (25%)	0	0
	<i>Lucilia</i> spp.	1 (12.5%)	0	0
	<i>H. fernandica</i>	1 (12.5%)	0	0
	Calliphoridae gen. sp.	0	1 (25%)	0
Sarcophagidae				
	Sarcophagidae gen. sp.	2 (25%)	0	0
Muscidae				
	<i>M. domestica</i>	1 (12.5%)	0	0
	<i>M. prolapsa</i>	1 (12.5%)	0	0
	<i>H. capensis</i>	0	1 (25%)	0
Piophilidae				
	Piophilidae gen. sp.	0	1 (25%)	1 (50%)
Sepsidae				
	Sepsidae gen. sp.	1 (12.5%)	0	0
Dermestidae				
	<i>D. maculatus</i> + Dermestidae gen. sp.	1 (12.5%)	2 (50%)	0
Cleridae				
	<i>N. rufipes</i> + Cleridae gen. sp.	0	4 (100%)	0
Histeridae				
	Histeridae gen. sp.	2 (25%)	1 (25%)	0
Staphylinidae				
	Staphylinidae gen. sp.	0	0	1 (50%)
Silphidae				
	<i>T. mutilatus</i>	1 (12.5%)	1 (25%)	0

5.3.5. Seasonal occurrence

Chrysomya albiceps and *L. sericata* (66.7%, 2 out of the 3 cases for each species) and *T. mutilatus* (66.7%, 2 out of the 3 cases) were the most frequently collected Calliphorid flies and beetles, respectively from decomposing human remains discovered in the spring season (Table 5.3, Tables D1 & D2). In the summer season, *Ch. albiceps* (71.4%, 5 out of 7 cases), *Ch. marginalis* (57.1%, 4 out of 7 cases) and members of the family Cleridae (28.6%, 2 out of the 7 cases) were the most frequently collected Calliphorid flies and beetles. Similarly, both Calliphorid fly species (i.e., *Ch. albiceps* and *Ch. marginalis*; 100%, 2 out of 2 for each species), and members of the family Dermestidae (100%, 2 out of the 2 cases) were the most common Calliphorid flies and beetles collected from decomposing human remains discovered in the autumn season. In the winter season, *Ch. chloropyga*, *Ch. albiceps* and *Ca. vicina* were collected from 50% (1 out of 2 cases) of the discovered human remains respectively. Within the same season, members of the family Staphylinidae were collected from 50% (1 out of 2 cases) of the discovered human remains. Members of other Dipteran families such as Sarcophagidae, Muscidae, Piophilidae and Sepsidae were scanty in their frequencies on decomposing human remains across the four seasons.

Table 5.3: Case frequency of insect species collected from 14 human forensic cases (n = number of cases) in different seasons within the Western Cape Province of South Africa from 2015 to 2022.

		Season (n = 14)			
Family	Species	Spring (n = 3; 21.4%)	Summer (n = 7; 50%)	Autumn (n = 2; 14.3%)	Winter (n = 2; 14.3%)
Calliphoridae					
	<i>Ch. chloropyga</i>	1 (33.3%)	2 (28.6%)	1 (50%)	1 (50%)
	<i>Ch. albiceps</i>	2 (66.7%)	5 (71.4%)	2 (100%)	1 (50%)
	<i>Ch. megacephala</i>	0	1 (14.3%)	0	0
	<i>Ch. marginalis</i>	1 (33.3%)	4 (57.1%)	2 (100%)	0
	<i>Ca. vicina</i>	0	0	0	1 (50%)
	<i>L. sericata</i>	1 (33.3%)	1 (14.3%)	0	0
	<i>L. cuprina</i>	0	2 (28.6%)	0	0
	<i>Lucilia</i> spp.	1 (33.3%)	0	0	0
	<i>H. fernanidca</i>	0	1 (14.3%)	0	0
	Calliphoridae gen. sp.	0	1 (14.3%)	0	0
Sarcophagidae					
	Sarcophagidae gen. sp.	0	1 (14.3%)	1 (50%)	0
Muscidae					
	<i>M. domestica</i>	1 (33.3%)	0	0	0
	<i>M. prolapsa</i>	0	0	0	1 (50%)
	<i>H. capensis</i>	0	0	1 (50%)	0
Piophilidae					
	Piophilidae gen. sp.	0	1 (14.3%)	0	1 (50%)
Sepsidae					
	Sepsidae gen. sp.	1 (33.3%)	0	0	0
Dermestidae					
	<i>D. maculatus</i> + Dermestidae gen. sp.	0	1 (14.3%)	2 (100%)	0
Cleridae					
	<i>N. rufipes</i> + Cleridae gen. sp.	1 (33.3%)	2 (28.6%)	1 (50%)	0
Histeridae					
	Histeridae gen. sp.	1 (33.3%)	1 (14.3%)	1 (50%)	0
Staphylinidae					
	Staphylinidae gen. sp.	0	0	0	1 (50%)
Silphidae					
	<i>T. mutilatus</i>	2 (66.7%)	0	0	0

5.4. Discussion

Although the sample of cases was relatively small, the cases reviewed provided valuable information on the insect species associated with decomposing human remains across several seasons. There were substantial similarities between the insects of forensic importance feeding and depositing their eggs/larvae on the pig carcasses used in the field trials and those collected from decomposing human remains within the Western Cape Province (Table D2). This observation supports the importance of using animal subjects (e.g., pigs) in generating baseline data on carrion-associated entomofauna in a given locality or region. Furthermore, it is consistent with the findings of other authors who found remarkably similar insect species of forensic importance on decomposing human cadavers used in field-based successional studies and those previously documented in actual forensic case works in Australia (Dawson *et al.*, 2021b).

General case evaluation

In the cases assessed by CapeFORTE, more cadavers were discovered decomposing in outdoor scenes when compared to those found indoors. This is in agreement with the findings of other similar studies (Sukontanson *et al.*, 2007; Baliso *et al.*, 2023). In contrast, other authors elsewhere found more decomposing human remains in indoor environments (Kumara *et al.*, 2012; Al-Khalifa *et al.*, 2020; Lutz *et al.*, 2021a; Yan *et al.*, 2023). In accordance with the findings of other authors (Sukontanson *et al.*, 2007; Talebzadeh *et al.*, 2017; Baliso *et al.*, 2019; Lutz *et al.*, 2021a; Yan *et al.*, 2023), a greater proportion of cases were male decedents. This was not unexpected, as males typically have a higher likelihood of unnatural deaths within the Western Cape Province (Reid *et al.*, 2020). Also, several authors have attributed the higher incidence of male decedents to their age, socio-economic status, place of residence, and the likelihood to interact with unknown individuals aggressively (Krug *et al.*, 2002; Otieno *et al.*, 2015; Clark *et al.*, 2017). A higher number of cases assessed by CapeFORTE consisted of human remains found decomposing in the summer season. This observation is not surprising as several studies within South Africa and elsewhere have reported the widespread occurrence of human remains in the summer season (Maistry, 2015; Lutz *et al.*, 2021a, b; Baliso *et al.*, 2019, 2023; Yan *et al.*, 2023).

Diptera

Members of the family Calliphoridae were the most common insects collected from the decomposing human remains. This observation agrees with the findings of other authors in

Malaysia, Thailand, and Taiwan (Sukontason *et al.*, 2007; Kavitha *et al.*, 2013; Yan *et al.*, 2023). *Chrysomya albiceps* was the most frequently occurring fly species on the human remains (Table 5.1). The adult and immature stages of this Calliphorid fly were found associated with human remains in various stages of decomposition (early decomposition to skeletonization) in both indoor and outdoor scenes across the autumn, summer, winter, and spring seasons (Tables 5.1 – 5.3). Similar observations were observed in Johannesburg, South Africa, where *Ch. albiceps* was reported to be the second most frequently occurring Calliphorid species on decomposing human cadavers (Hill, 2019). Furthermore, several other authors in various parts of the world have found *Ch. albiceps* to be the dominant blow fly species colonizing outdoor and indoor decomposing human cadavers (Oliviera and Vasconcelos, 2010; Talebzadeh *et al.*, 2017 and Al-Khalifa *et al.*, 2020).

In this study, *Ch. albiceps* were mostly found on human cadavers discovered in the summer season (Table 5.3). This observation is not unusual as *Ch. albiceps* is known to be a heat-loving blow fly species predominantly found occurring in the summer and autumn seasons of South Africa and other countries (Battan Horenstein *et al.*, 2007; Kelly *et al.*, 2011; Abd El-Bar and Sawaby, 2011; Gilbert, 2014; Sebastião and Prado e Castro, 2019; Zeariya and Kabadaia, 2019; Tembe and Mukaratirwa, 2021). In comparison to the field experimental studies using neonate and adult pig carcasses, *Ch. albiceps* was found to be the second most abundant blow fly species in the summer season of the Western Cape Province (Figures 3.13 – 3.15, 4.4). This blow fly species was also observed to oviposit on the neonate and adult pig carcasses during the summer seasonal period. In various stages of pig carcass decomposition across the autumn, spring, and winter seasonal trials, only the adults of *Ch. albiceps* were observed feeding on the neonate pig carcasses and the decomposition fluids seeping out from them. It is possible that season and its associated factors such as temperature, humidity and rainfall play a key role in controlling the oviposition behavior of blow flies (Oliva, 2001) and this might explain why the eggs of *Ch. albiceps* were not recorded on the neonate pig carcasses across the three seasonal trials.

In this study, the immature stages of *Ca. vicina* were found feeding on a human cadaver in the early stage of decomposition, discovered in an indoor environment in the winter season (Tables 5.1 – 5.3, D1 & D2). This observation is consistent with the findings of other authors in America and Europe. Sanford (2017) in the United States of America and Lutz *et al.* (2021a) in Germany reported *Ca. vicina* to be one of the most common Calliphorid flies that fed and deposited its eggs on decomposing human remains in indoor environments. Similarly, Kadej *et*

al. (2020) observed dead adults and empty puparia of *Ca. vicina* on a skeletonized human corpse in an indoor death scene. In Johannesburg, South Africa, *Ca. vicina* was reported to be the most commonly occurring Calliphoridae species on decomposing human cadavers autopsied at Forensic Pathology Services medico-legal laboratories in Gauteng (Hill, 2019). The occurrence of *Ca. vicina* and their oviposition on decomposing vertebrate remains in the winter season is not surprising as they are known to be a cold-adapted invasive blow fly species prevalent mostly in the winter season of South Africa.

In the field experimental studies on the insect species associated with decomposing neonate pigs within the vicinity of the Table Mountain National Park, the eggs, and larvae of *Ca. vicina* were recorded on the neonate pig carcasses in the spring and winter seasons (Figures B6, B40 & B41). Congruent with the field observations on neonate pig carcasses, Bonacci *et al.* (2009) recorded the immature stages of *Ca. vicina* on a human cadaver in the winter season of the City of Cosenza, Italy. The presence of the larvae of *Ca. vicina* on a human cadaver in the early decomposition stage in an indoor scene within the winter season as shown here, validates the findings of the winter season field decomposition and insect successional trials on neonate pig carcasses.

The adult and juvenile stages of *Ch. marginalis* were found associated with human cadavers in the early, advanced decomposition and skeletonization stages in both indoor and outdoor environments across the autumn, summer, and spring seasons (Tables 5.1 – 5.3, D1 & D2). The immature stages of *Ch. marginalis* were mostly found on human cadavers discovered in the summer season. This might be attributed to their preference for the summer season as shown in several studies in the summer season of South Africa (Braack, 1986; Richards *et al.*, 2009a, b; Kelly *et al.*, 2011). During the neonate pig experimental trials, the larvae of *Ch. marginalis* were seen feeding on a neonate pig carcass in the summer season. Similarly, *Ch. marginalis* successfully oviposited on adult pig carcasses deployed outdoors in the warm (i.e., summer, spring, and autumn) seasonal period of the Free State Province (Kelly *et al.*, 2011). Although it was initially suspected that the few numbers of *Ch. marginalis* adults and the entire absence of their eggs on the neonate pig carcasses in the spring, autumn, and winter seasonal trials, was due to the species suggested preference for large size carcasses (Richards *et al.*, 2009a, b; Sebastião and Prado e Castro, 2019). However, results from the adult pig summer trial alongside those of Finaughty (2019) seem to suggest that carcass size has no influence on the oviposition behavior of *Ch. marginalis*.

Chrysomya chloropyga is known to occur in all four seasons of South Africa (Kolver, 2009; Richards *et al.*, 2009a, b; Kelly *et al.*, 2008, 2011). Here, *Ch. chloropyga* was the third most frequently occurring fly species on the human cadavers (Table 5.1). Hill (2019) also found *Ch. chloropyga* to be one of the most common blow flies on decomposing human cadavers in Johannesburg. In this study, the adults, larvae, and pupae of *Ch. chloropyga* were recovered from human decedents in the early, advanced decomposition and skeletonization stages, in outdoor environments across the four seasons of South Africa (Tables 5.1 – 5.3, D1 & D2). In the neonate pig trials within the Table Mountain National Park, the eggs, and larvae of *Ch. chloropyga* were recorded on the pig carcasses in the spring, summer, autumn, and winter seasonal trials (Figures B6, B8, B9, B11, B17, B21, B28 – B30, B41, B44). *Chrysomya chloropyga* was found to be the most dominant and abundant blow fly species in the spring and winter seasons.

The larvae of *Ch. megacephala* were found feeding on a human cadaver in the advanced decomposition stage in an outdoor environment during the summer season (Tables 5.1 – 5.3, D1 & D2). In the field experimental studies, the adults of *Ch. megacephala* were consistently recorded feeding and interacting with decomposing neonate pig carcasses in the summer, autumn, spring, and winter seasons (Figures B6 – B11, B17 – B22, B28 – B33, B39 – B44). In addition, *Ch. megacephala* was found to be the most abundant and dominant fly in the neonate pig summer season trials and their eggs and larvae were recorded on the neonate pig carcasses in the summer season. The presence of the *Ch. megacephala* on the decomposing human cadaver in an outdoor environment and in the summer season as shown here is both in agreement with the findings of the neonate pig trials and that of other authors who found *Ch. megacephala* to be one of the most frequently occurring Calliphorid species on outdoor decomposing human cadavers in the summer season of Taiwan (Yan *et al.*, 2023). Other authors have found *Ch. megacephala* to be the most commonly occurring blow fly species on human cadavers in both outdoor and indoor environments in Malaysia and India, respectively (Kumara *et al.*, 2012; Palavesam *et al.*, 2022).

In this study, the larval and adult stages of *L. sericata* and *L. cuprina* were found in close association of human cadavers in the early decomposition stage, in both indoor and outdoor environments in the spring and summer seasons (Tables 5.1 – 5.3, D1 & D2). In the field studies, the abundance of both *Lucilia* species across the four seasons was significantly low on decomposing neonate and adult pig carcasses. Furthermore, the eggs of both *Lucilia* species were not observed on the neonate and adult pig carcasses in any of the seasonal trials.

In contrast, Kelly *et al.* (2011) found both the adults and larvae of *Lucilia* species on decomposing adult pig carcasses in the warm season of the Free State Province. The larvae of *H. fernandica* were recovered from an indoor decomposing human cadaver at the early decomposition stage in the summer season. The presence of the larvae of *H. fernandica* on the decomposing human cadaver is in agreement with the observations recorded during the neonate pig trials and those of Meskin (1986). During the early and advanced decomposition stages, the eggs and larvae of *H. fernandica* were recorded on the neonate pig carcasses in the summer and autumn seasons (Figures B22, B30, B32 & B33).

The larval and adult stages of Sarcophagidae species were recovered from outdoor and indoor decomposing human cadavers in the early decomposition stage, in the autumn and summer seasons respectively (Tables 5.1 – 5.3, D1 & D2). In the field experimental studies, the larval and adult stages of Sarcophagidae species were found on the neonate pig carcasses in the early and advanced decomposition stages across the spring, summer, autumn and winter seasons (Figures B7, B9 – B11, B17 – B22, B28, B31, & B41). The presence of the adult and larval stages of Sarcophagidae species on decomposing human cadavers in both indoor and outdoor scenes further agrees with the findings documented in the field studies on decomposing neonate pigs. Other authors have reported several members of the family Sarcophagidae breeding on decomposing human corpses in both indoor and outdoor environments (Sanford, 2017; Yan *et al.*, 2023). However, the application of Sarcophagidae species in minimum time since death estimations has had been challenging. This is due to the difficulty associated with their accurate species identification and lack of sufficient developmental data (Cherix *et al.*, 2012; Sanford, 2017).

The larvae of *Hydrotaea capensis* were recovered from a partly skeletonized human cadaver in an outdoor scene in the autumn season (Tables 5.1 – 5.3, D1 & D2). During the field studies, only the adult stages of *Hydrotaea* sp. were observed feeding and hovering above the neonate and adult pig carcasses mostly during the advanced decomposition and skeletonization stages across the four seasonal cycles (Figures B6 – B11, B17 – B22, B28 – B33, B39 – B44, 4.6 & 4.12). Lutz *et al.* (2021a) reported that *Hydrotaea* species demonstrated a preference for cadavers decomposing in outdoor scenes. In this study, the larvae of *Muscina prolapsa* were recovered from an indoor decomposing human cadaver in the early decomposition stage during the winter season (Tables 5.1 – 5.3). According to Lutz *et al.* (2021a), the presence of *Muscina* species including *M. prolapsa* on vertebrate remains can serve as valuable forensic indicators of neglect, corpse concealment and burial, agreeing with the earlier reports by Smith (1986)

and Gunn (2016) who noted the adults and larvae of *Muscina* species on feces and buried baits. The adult stage of *M. domestica* was recovered from an indoor decomposing human cadaver in the early decomposition stage in the spring season (Tables 5.1 – 5.3). Several *Musca* sp. adult individuals were observed on the decomposing neonate and adult pig carcasses in all the seasonal trials (Figures B6 – B11, B17 – B22, B28 – B33, B39 – B44, 4.6 & 4.12).

Members of the family Sepsidae were recovered from an outdoor decomposing human cadaver in the early stage of decomposition in the spring season (Tables 5.1 – 5.3). In all the spring season neonate pig trials, the adult but not the immature stages of Sepsidae species were found on the carcasses during the early and advanced decomposition stages (Figures B7 – B11, B17 – B22, B28 – B33, B39 – B42). In contrast to Sepsidae, the larvae and pupae of Piophilidae species were recovered from outdoor decomposing human remains in the advanced decomposition and skeletonization stages during the summer and winter seasons respectively (Tables 5.1 – 5.3). This observation is not unusual as Piophilidae species have been reported to exhibit a preference for dry proteinaceous food substances and extremely decomposed animal carcasses usually in their late stages of decomposition (Byrd and Castner, 2010; Heo *et al.*, 2011). In the field studies, the adults and not the larvae/pupae of Piophilid species were observed on the neonate and adult pig carcasses mostly during the late stages of decomposition in all the trials.

Coleoptera

Members of the family Dermestidae are usually of significant forensic importance especially in the estimation of the PMImins of forensic cases with extended PMIs (Lutz *et al.*, 2021a). The larval and adult stages of Dermestid beetles are often found clustering and feeding on the dried skin and mummified tissues of decomposing human and animal remains in the late early, advanced decomposition and skeletonization stages (Smith, 1986; Abd El-Bar *et al.* 2016; Bonacci *et al.*, 2017; Kadej *et al.*, 2020, 2022; Ries *et al.*, 2021; Lutz *et al.*, 2021a). In this study, specifically in the autumn season, the adults of Dermestidae species were recovered from outdoor decomposing human remains in the early and advanced decomposition stages, while both the adults and larvae were found on just one human cadaver in the advanced decomposition stage in the summer season (Tables 5.1 – 5.3, D1 & D2).

During the autumn and summer field trials on neonate pigs, several adult Dermestid beetles were observed to arrive on the neonate pig carcasses following their transition into the advanced decomposition and skeletonization stages. Similar observations were recorded during

the summer adult pig trial (Figure 4.6). However, towards the end of the advanced decomposition stage of the adult pig, several larvae of Dermestid beetles were observed. In contrast, during the early and mid-spring seasonal trials, adult Dermestid beetles were observed to arrive on neonate pig carcasses during the early decomposition stage. The presence of the adults and larvae of Dermestid beetles on the human remains that are partly mummified and in the early/advanced decomposition stage in the summer and autumn seasons within this region, is in agreement with the findings of the autumn and summer decomposition trials on the neonate and adult pig carcasses.

Adult Cleridae species including *Necrobia rufipes* (Fabricius, 1781) were collected from outdoor decomposing human remains in the advanced decomposition stage within the autumn, spring, and summer seasons (Tables 5.1 – 5.3). As with other forensically important beetle species, they are often attracted to decomposing vertebrate remains in the late stages of decomposition. Adult Clerid beetles were only seen during the neonate pig summer trials, with very few individuals recorded mostly during the advanced decomposition and skeletonization stages (Figures B18, B19, & B21). In contrast, more Clerid beetles were recorded during the adult pig summer trial (Figure 4.6). Forensic entomological reports from other countries have also documented the presence of the adult and larval stages of Clerid beetles on decomposing human cadavers (Shin *et al.*, 2015; Charabidze *et al.*, 2016; Bonacci *et al.*, 2017; Bajerlein *et al.*, 2018; Lutz *et al.*, 2021a; Maisonhaute and Forbes, 2023). The presence of adult *N. rufipes* on the human remains, especially in the summer season as shown here, are in agreement with the findings of the summer field trials on neonate and adult pig carcasses. Furthermore, the occurrence of *N. rufipes* on human remains during the spring season is in line with the observations recorded during the adult pig winter trial where Clerid beetles were observed to persist on the carcass till the late spring seasonal period.

In this study, specifically in the spring season, only the adults of *Thanatophilus mutilatus* (Castelneau, 1840) were recovered from outdoor decomposing human cadavers in the early and advanced decomposition stages (Tables 5.1 – 5.3). This is in line with the findings of other authors who sampled *Necrodes littoralis* (Linnaeus, 1758) from human cadavers mostly in the early and advanced decomposition stages (Charabidze *et al.*, 2016). The authors noted the preference of *N. littoralis* for outdoor decomposing cadavers and further revealed that most of the cases from which *N. littoralis* were collected occurred during the spring and summer seasons. In the early and mid-spring neonate pig trials, several adult Silphid beetles were observed to arrive and colonize the decomposing pig carcasses during the early and late

decomposition stages. In the adult pig winter trial, Silphid beetles were observed to arrive on the pig carcass during the early and late decomposition stages until the termination of the trial in the late spring period (Figure 4.12). Several other authors have documented the presence of the adult and larval stages of several Silphid beetles on human cadavers at different stages of decomposition (Shin *et al.*, 2015; Bajerlein *et al.*, 2018; Maisonhaute and Forbes, 2023).

The adults of Histeridae species were recovered from outdoor decomposing human remains in the early decomposition and advanced decomposition stages across the spring, summer, and autumn seasons (Tables 5.1 – 5.3). The occurrence of Histeridae species on vertebrate remains during the early decomposition stage is not unusual. During the field neonate pig decomposition trials in the spring, summer and autumn seasons, several adults of Histeridae species were observed to arrive on the neonate pig carcasses during the early and advanced decomposition stages presumably in search for fly eggs and larvae for food. They were further observed to persist on the carcasses until the skeletonization stage. This was the case in the adult pig summer trial (Figure 4.6).

The presence of the members of the family Histeridae on human cadavers in varied decomposition states have been reported by several authors from various parts of the world (Shin *et al.*, 2015; Charabidze *et al.*, 2016; Bajerlein *et al.*, 2018; Maisonhaute and Forbes, 2023). The adults of Staphylinidae species were recovered from an outdoor human cadaver in the skeletonization stage in the winter season (Tables 5.1 – 5.3). In the neonate pig winter trials, adult Staphylinidae species were recorded on the pig carcasses, arriving mostly during the advanced decomposition and skeletonization stages. Other authors have similarly reported the occurrence of the members of the family Staphylinidae on human cadavers in varied decomposition states (Moemembellah-Fard *et al.*, 2018; Charabidze *et al.*, 2016; Maisonhaute and Forbes, 2023).

Collection of entomological specimens from the crime scene and their utilization in PMImin. estimation within the Western Cape Province

An interesting observation was the limited number of cases [(n = 3, out of a total of 3 crime/death scenes' collection), see section 5.3.1.] where an entomologist collected insects from the crime/death scene. This is due to the likelihood of a forensic entomologist attending a crime or death scene and autopsy sessions which is often at the discretion of the attendant forensic pathologists. This observation is worrisome, and it supports the notion by Lutz *et al.* (2021a) and Niederegger and Mall (2021) that the potential value of forensic entomology in

PMI estimations is considerably undermined. Furthermore, in the Regulations Regarding the Rendering of Forensic Pathology Services in South Africa (South Africa National Health Act No. 61 of 2003), it is clearly stated that the forensic pathologists may consult other professionals and request for their participation and expertise during the post-mortem analysis of the deceased body either at the scene of death and during autopsy. Consequently, it is not out of place to speculate that the above-mentioned discretionary powers of the attendant forensic pathologists may have contributed to the considerably low number of actual forensic cases with entomological evidence assessed by members of CapeFORTE. Furthermore, this is not a good practice in forensic science as any object associated with the crime scene can be used as evidence as long as they provide valuable information on the activities connected with the crime scene or crime (Metcalf *et al.*, 2013; Lutz *et al.*, 2021a). Baliso *et al.* (2023) reported the occurrence of entomological evidence in nearly half (i.e., 76 out of 174 cases) of the actual forensic anthropological cases they analyzed within the Western Cape Province. Furthermore, Baliso *et al.* (2023) revealed that the occurrence of entomological evidence improved the estimation of the PMI with narrower ranges echoing the findings of Lutz *et al.* (2021a).

The results from this study revealed that half of the cases assessed by CapeFORTE had their associated entomological evidence collected by the attendant pathologists. A closer look at the contents of the entomological reports revealed that some of the specimens collected by the pathologists were killed/preserved by direct placement in solvents like water, ethanol, and formalin. In addition, some of the entomological specimens collected by the pathologists were kept alive, unfed, packaged in sealed screw top containers without aeration and transported to the CapeFORTE laboratory for analysis. These observations indicate a need for the training of pathologists and forensic pathology officers in the study region in relation to the collection and preservation of cadaver-associated entomological specimens as documented in Amendt *et al.* (2007). Consequently, it was not unusual in those cases that most of the entomological specimens were not useful in the estimation of the PMImin owing to the degradation and shrinkage of the specimens. This observation agrees with some of the points made by Matuszewski (2021) regarding the collection and preservation of entomological specimens by non-experts.

A large percentage of the cases assessed by CapeFORTE had their associated entomological specimens collected during autopsy and a few collected during autopsy and at the scene of death. This observation is consistent with the findings of Sanford (2017). In some instances, the collection of entomological specimens at the crime scene may not be possible

until autopsy due to several reasons including but not limited to the timing and availability of forensic entomologists, lack of resources for transport, and safety concerns in and around the scenes (Baliso *et al.*, 2023). Amid the removal from the crime scene and autopsy, human remains are sometimes cleaned, and stored in a refrigerator or freezer for some hours or days (Lutz *et al.*, 2021b; Baliso *et al.*, 2023). The act of cleaning some of the human remains prior to autopsy as suggested by Baliso *et al.* (2023) may bring about the loss of valuable entomological evidence that can assist in the resolution of the PMImin. Furthermore, the question of whether refrigeration or freezing of the cadaver before autopsy exerts an impact on the development of its associated fly larvae has caused some debate in forensic entomology (Johl and Anderson, 1996; Myskowiak and Doums, 2002; Magni *et al.*, 2016; Archer *et al.*, 2018; Lutz *et al.*, 2021b). However, according to Huntington *et al.* (2007), the assumption that no maggot development occurs during the refrigeration process of a decomposing vertebrate remains prior to autopsy could lead to an error rate between 8.6 – 12.8% during PMI estimations. Additionally, the process of corpse refrigeration together with the maggot masses could potentially jeopardize certain evidence on the corpse due to the continuous development and feeding activities of the maggots on the soft tissues of the body (Huntington *et al.*, 2007; Magni *et al.*, 2016).

Therefore, as highlighted by Lutz *et al.* (2021b), it is important to ensure that a forensic entomologist (or someone with appropriate training) is the primary collector of the cadaver-associated entomological specimens at the scene of death and during autopsy. Admittedly, entomological sampling at the death scene may be impossible in some instances due to some of the issues mentioned above, however, this should be done immediately following the arrival of the corpse at the mortuary and it should not be limited to the body alone but also other associated materials such as the body bag, and clothing materials associated with the body (Lutz *et al.* 2021b). Above all, the utilization of entomological samples collected during autopsy should be used with caution in PMImin estimations.

Chapter 6: Changes in soil pH and electrical conductivity, skin pH, and larval mass pH – Neonate and Adult Pig Decomposition Trials

6.1. Neonate pig trials (All seasons)

6.1.1. Soil pH

In all the trials, carcass soil pH was significantly higher than the control soil samples with an overall trend of increasing pH as decomposition progressed (Table 6.1). Generally, the decomposition stage of the carcass had a significant impact on the pH of the carcass soil with significantly greater pH recorded during the advanced decomposition and skeletonization stages compared to the fresh and early decomposition stages (Table 6.2).

Table 6.1: Statistical summary of Wilcoxon signed-rank test ($p < 0.05$) used to determine significant differences between carcass and control soil pH in each monthly neonate pig decomposition trial across the spring, summer, autumn and winter seasons.

Soil pH					
Trial	Source	Spring	Summer	Autumn	Winter
Early	Carcass	7.7 ± 1.0	6.6 ± 0.8	8.1 ± 1.2	8.1 ± 0.8
	Control	6.3 ± 0.2	5.9 ± 0.3	6.7 ± 0.3	5.6 ± 0.4
	<i>p</i> value	< 0.0001	0.009	< 0.001	< 0.0001
Mid	Carcass	6.6 ± 0.6	7.5 ± 1.4	8.2 ± 1.0	7.9 ± 1.2
	Control	6.3 ± 0.2	6.6 ± 0.4	6.4 ± 0.4	5.7 ± 0.3
	<i>p</i> value	0.02	0.04	0.0001	< 0.0001
Late	Carcass	7.3 ± 1.4	7.7 ± 1.2	8.1 ± 1.0	6.9 ± 0.7
	Control	6.3 ± 0.3	6.6 ± 0.2	6.0 ± 0.3	6.1 ± 0.4
	<i>p</i> value	0.03	0.008	0.0001	< 0.0001

Values indicate mean $\pm$ standard deviation.

Table 6.2: Statistical summary of carcass soil pH across the four decomposition stages in each monthly neonate pig decomposition trial in the spring, summer, autumn and winter seasons. Means $\pm$ standard deviation within each column followed by the different letters are significantly different after a Tukey HSD or Dunn's test ($p < 0.05$). Overall p value indicates the statistical significance of the model for each trial after a one-way ANOVA or Kruskal-Wallis rank sum test.

Trial	Decomposition stage				
	F	ED	AD	SK	Overall p value
Spring					
Early	5.9 $\pm$ 0.04 ^b	7.4 $\pm$ 0.91 ^{ab}	8.3 $\pm$ 0.6 ^a	8.4 $\pm$ 0.4 ^a	$p = 0.002$
Mid	6.4 $\pm$ 0.06 ^{ab}	6.2 $\pm$ 0.5 ^b	6.7 $\pm$ 0.7 ^{ab}	7.1 $\pm$ 0.3 ^a	$p = 0.014$
Late	6.0 $\pm$ 0.1 ^{ab}	6.1 $\pm$ 1.1 ^a	7.7 $\pm$ 1.0 ^{ab}	8.6 $\pm$ 0.4 ^b	$p = 0.001$
Summer					
Early	5.5 $\pm$ 0.4 ^b	6.2 $\pm$ 0.9 ^{ab}	6.8 $\pm$ 0.8 ^{ab}	7.2 $\pm$ 0.5 ^a	$p = 0.028$
Mid	6.1 $\pm$ 0.0 ^{ab}	6.2 $\pm$ 0.9 ^b	8.6 $\pm$ 0.2 ^a	8.7 $\pm$ 0.2 ^a	$p < 0.001$
Late	6.7 $\pm$ 0.7 ^{ab}	6.6 $\pm$ 1.2 ^a	8.4 $\pm$ 0.4 ^{ab}	8.7 $\pm$ 0.2 ^b	$p = 0.002$
Autumn					
Early	6.4 $\pm$ 0.1 ^{ab}	7.3 $\pm$ 1.2 ^b	9.0 $\pm$ 0.1 ^a	8.9 $\pm$ 0.3 ^a	$p < 0.001$
Mid	6.4 $\pm$ 0.1 ^{ab}	7.6 $\pm$ 1.2 ^b	8.8 $\pm$ 0.3 ^a	8.8 $\pm$ 0.2 ^{ab}	$p = 0.002$
Late	5.7 $\pm$ 0.3 ^b	8.0 $\pm$ 1.0 ^a	8.5 $\pm$ 0.4 ^a	8.8 $\pm$ 0.4 ^a	$p < 0.001$
Winter					
Early	6.2 $\pm$ 0.1 ^b	7.7 $\pm$ 0.8 ^b	8.6 $\pm$ 0.3 ^a	8.6 $\pm$ 0.2 ^a	$p < 0.0001$
Mid	5.4 $\pm$ 0.2 ^b	7.5 $\pm$ 1.3 ^b	8.7 $\pm$ 0.2 ^a	8.8 $\pm$ 0.3 ^a	$p < 0.0001$
Late	6.3 $\pm$ 0.3 ^b	6.5 $\pm$ 0.3 ^b	7.2 $\pm$ 0.5 ^a	7.5 $\pm$ 1.1 ^a	$p < 0.0001$

F = Fresh; ED = Early decomposition; AD = Advanced decomposition; SK = Skeletonization.

In the summer and autumn seasonal trials, mean carcass soil pH began to increase steadily between Days 3 and 4, with an acid to alkaline shift in carcass soil pH noted between Days 4 and 6. The carcass soil was largely an alkaline environment until the termination of the trials in these two seasons (Figure 6.1). In contrast, in the spring and winter seasonal trials, there was a considerable variation in the transition days of the carcass soil pH from an acidic state to an alkaline state (Figure 6.1).

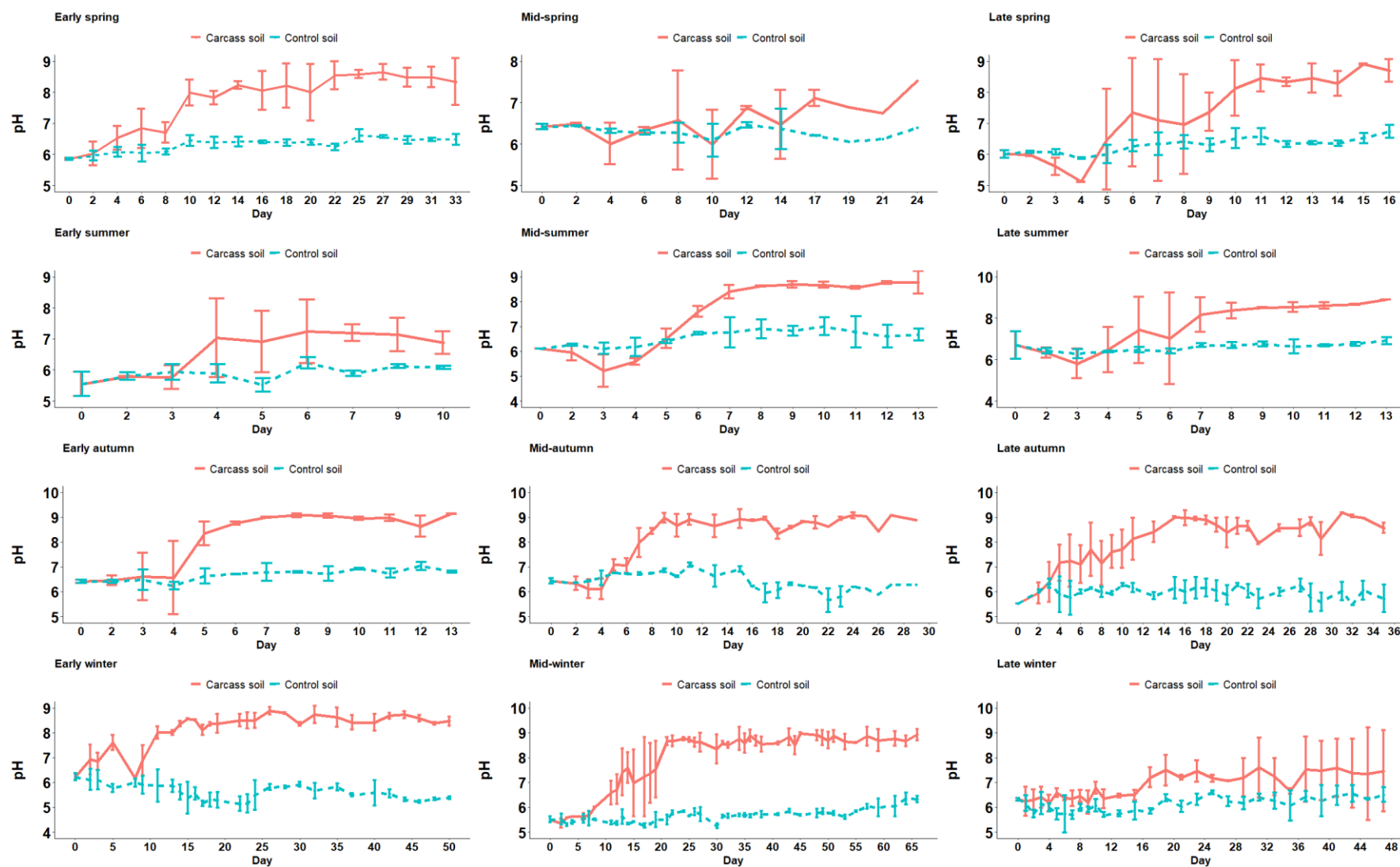


Figure 6.1: Daily mean pH of carcass (red solid lines) and control (blue broken lines) soils in each month across the spring, summer, autumn, and winter seasonal periods. Error bars represent standard deviations where $n = 2$.

6.1.2. Soil electrical conductivity

In all the trials, the electrical conductivity of the carcass soil was significantly higher than the control soil (Table 6.3). Apart from the mid-spring and mid-autumn trials, the electrical conductivity of the carcass soil was significantly impacted by the decomposition stage of the carcass (Table 6.4). Generally, there were differences in the trends of the carcass soil electrical conductivity between the seasonal trials and most increments were noticeable during the early and advanced decomposition stages of the carcasses (Figure 6.2).

Table 6.3: Statistical summary of Wilcoxon signed-rank test ($p < 0.05$) used to determine significant differences between carcass and control soil electrical conductivity ($\mu\text{S}/\text{cm}$) in each monthly neonate pig decomposition trial across the spring, summer, autumn and winter seasons.

Soil Electrical conductivity					
Trial	Source	Spring	Summer	Autumn	Winter
Early	Carcass	705 $\pm$ 505.7	855.5 $\pm$ 543.8	1513.8 $\pm$ 835.6	502 $\pm$ 286.7
	Control	166.9 $\pm$ 14.6	141.3 $\pm$ 15.1	178.6 $\pm$ 32.3	133 $\pm$ 7.5
	<i>p</i> value	< 0.0001	< 0.001	< 0.0001	< 0.0001
Mid	Carcass	546.7 $\pm$ 489.9	1648.1 $\pm$ 843.2	806.4 $\pm$ 403.2	573.6 $\pm$ 339.7
	Control	185.3 $\pm$ 48.7	171.7 $\pm$ 19.0	144.1 $\pm$ 12.6	129.1 $\pm$ 5.9
	<i>p</i> value	< 0.001	< 0.0001	< 0.0001	< 0.0001
Late	Carcass	1038.3 $\pm$ 561.7	1589.6 $\pm$ 824.2	615.5 $\pm$ 289.3	411.8 $\pm$ 334.8
	Control	150.6 $\pm$ 11.3	154.6 $\pm$ 22.1	142.6 $\pm$ 23.4	151.7 $\pm$ 54.1
	<i>p</i> value	< 0.0001	< 0.0001	$p < 0.0001$	< 0.0001

Values indicate mean $\pm$ standard deviation.

Table 6.4: Statistical summary of carcass soil electrical conductivity ($\mu\text{S}/\text{cm}$) across the four decomposition stages in each monthly neonate pig decomposition trial across the spring, summer, autumn and winter seasons. Means $\pm$ standard deviation within each column followed by the different letters are significantly different after a Tukey HSD or Dunn's test ($p < 0.05$). Overall p value indicates the statistical significance of the model for each trial after a one-way ANOVA or Kruskal-Wallis rank sum test.

Trial	Decomposition stage				
	F	ED	AD	SK	Overall p value
Spring					
Early	152.6 $\pm$ 3.7 ^a	462.4 $\pm$ 300.8 ^a	696.9 $\pm$ 267.8 ^{ab}	1461.6 $\pm$ 380.7 ^b	$p < 0.001$
Mid	183.9 $\pm$ 1.8 ^a	479.7 $\pm$ 466.1 ^a	939.9 $\pm$ 803.5 ^a	506 $\pm$ 197.2 ^a	$p = 0.14$
Late	164.4 $\pm$ 2.9 ^b	1368.9 $\pm$ 692.8 ^a	1025.9 $\pm$ 398.9 ^{ab}	862.3 $\pm$ 346.6 ^{ab}	$p = 0.02$
Summer					
Early	113.7 $\pm$ 25 ^b	662.6 $\pm$ 563.2 ^{ab}	1445 $\pm$ 186.3 ^a	930.2 $\pm$ 386.6 ^{ab}	$p = 0.021$
Mid	172.6 $\pm$ 30.1 ^b	1083.8 $\pm$ 452.3 ^b	2175.5 $\pm$ 441.1 ^a	2327 $\pm$ 543 ^a	$p < 0.0001$
Late	180.8 $\pm$ 75 ^b	1169.3 $\pm$ 711 ^b	2274.5 $\pm$ 499.3 ^a	1953.4 $\pm$ 474.1 ^a	$p < 0.001$
Autumn					
Early	144.6 $\pm$ 8.5 ^b	1191.8 $\pm$ 640.3 ^b	1488.7 $\pm$ 282.2 ^{ab}	2277.5 $\pm$ 747.5 ^a	$p < 0.001$
Mid	170.3 $\pm$ 7.4 ^a	802.4 $\pm$ 262.6 ^a	902.7 $\pm$ 525.9 ^a	758.6 $\pm$ 300.5 ^a	$p = 0.12$
Late	145.2 $\pm$ 6.3 ^b	631.8 $\pm$ 281.6 ^a	669.2 $\pm$ 296.8 ^a	682.2 $\pm$ 211.4 ^a	$p = 0.01$
Winter					
Early	155.7 $\pm$ 16.8 ^a	414.7 $\pm$ 333.9 ^a	527.3 $\pm$ 165.5 ^{ab}	771.9 $\pm$ 174.7 ^b	$p < 0.001$
Mid	142.6 $\pm$ 8 ^b	518.9 $\pm$ 321.5 ^a	586.1 $\pm$ 132.2 ^a	976.4 $\pm$ 498.1 ^c	$p < 0.0001$
Late	65.2 $\pm$ 46.3 ^b	269.8 $\pm$ 278.3 ^b	550.7 $\pm$ 260.4 ^a	670.8 $\pm$ 359.3 ^a	$p < 0.0001$

F = Fresh; ED = Early decomposition; AD = Advanced decomposition; SK = Skeletonization; Values indicate mean $\pm$ standard deviation.

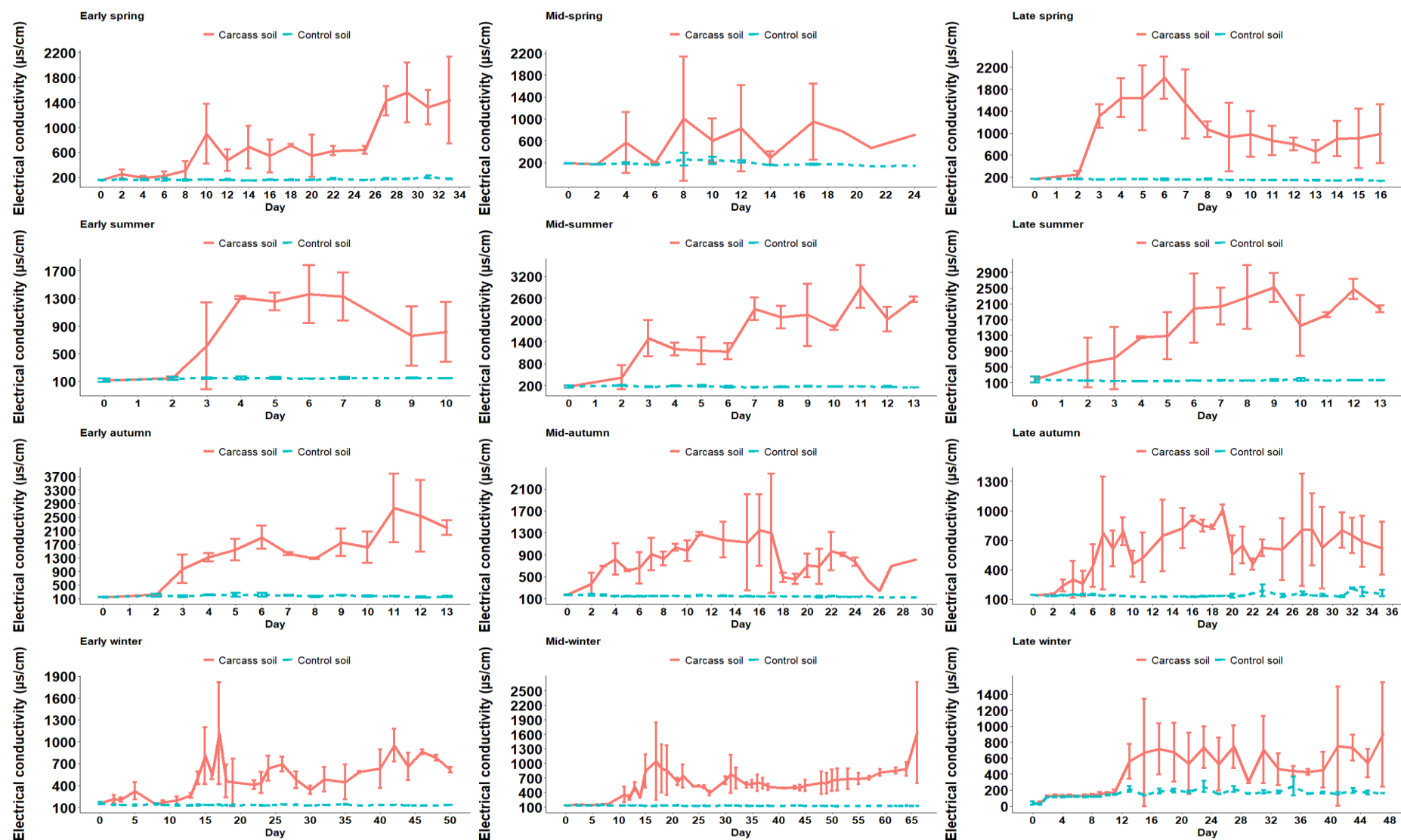


Figure 6.2: Daily mean electrical conductivity of carcass (red solid lines) and control (blue broken lines) soils in each month across the spring, summer, autumn, and winter seasonal periods. Error bars represent standard deviations where $n = 2$.

6.1.3. Carcass skin pH

Except for the mid-spring trial, the decomposition stage of the carcass had a significant impact on the pH of the carcass skin with significantly higher pH recorded during the advanced decomposition and skeletonization stages (Table 6.5). Overall, there was a high variability in carcass skin pH in all the seasonal trials with carcass skin pH increasing steadily as decomposition progressed albeit fluctuating between acidity and alkalinity on some days (Figure 6.3).

Table 6.5: Statistical summary of carcass skin pH across the four decomposition stages in each monthly neonate pig decomposition trial across the spring, summer, autumn and winter seasons. Means $\pm$ standard deviation within each column followed by the different letters are significantly different after a Tukey HSD or Dunn's test ($p < 0.05$). Overall p value indicates the statistical significance of the model for each trial after a one-way ANOVA or Kruskal-Wallis rank sum test.

Trial	Decomposition stage				Overall <i>p</i> value
	F	ED	AD	SK	
Spring					
Early	4.6 ± 0.1 ^b	5.9 ± 1.2 ^b	7.3 ± 0.7 ^a	7.9 ± 1.1 ^a	<i>p</i> < 0.0001
Mid	6.5 ± 0.8 ^a	6.6 ± 1.1 ^a	7.3 ± 1.4 ^a	7.7 ± 1.2 ^a	<i>p</i> = 0.09
Late	5.8 ± 0.3 ^{ab}	5.4 ± 0.4 ^a	6.1 ± 1.4 ^a	8.1 ± 1.0 ^b	<i>p</i> = 0.002
Summer					
Early	5.3 ± 0.0 ^a	5.1 ± 0.2 ^a	6.6 ± 1.2 ^a	7.2 ± 0.1 ^a	<i>p</i> = 0.03
Mid	5.2 ± 0.2 ^a	5.9 ± 1.0 ^a	6.2 ± 1.1 ^a	8.5 ± 0.4 ^b	<i>p</i> = 0.001
Late	-	6.0 ± 1.0 ^b	7.8 ± 1.0 ^a	8.5 ± 0.8 ^a	<i>p</i> < 0.0001
Autumn					
Early	5.8 ± 0.07 ^a	6.0 ± 0.8 ^a	7.3 ± 0.8 ^{ab}	9.2 ± 0.6 ^b	<i>p</i> < 0.001
Mid	5.8 ± 0.3 ^{ab}	5.5 ± 0.5 ^b	6.9 ± 1.1 ^a	7.1 ± 1.7 ^{ab}	<i>p</i> = 0.002
Late	5.5 ± 0.2 ^{ac}	5.9 ± 0.8 ^c	7.0 ± 0.8 ^{ab}	8.0 ± 0.6 ^b	<i>p</i> < 0.0001
Winter					
Early	5.3 ± 0.04 ^{ac}	5.5 ± 0.7 ^c	7.0 ± 0.6 ^{ab}	7.9 ± 0.6 ^b	<i>p</i> < 0.0001
Mid	4.8 ± 0.1 ^b	5.2 ± 0.6 ^b	6.9 ± 0.7 ^a	8.6 ± 0.5 ^a	<i>p</i> < 0.0001
Late	5.1 ± 0.2 ^{ab}	4.8 ± 0.8 ^a	5.6 ± 1.3 ^a	7.4 ± 1.4 ^b	<i>p</i> < 0.0001

F = Fresh; ED = Early decomposition; AD = Advanced decomposition; SK = Skeletonization; Values indicate mean $\pm$ standard deviation.

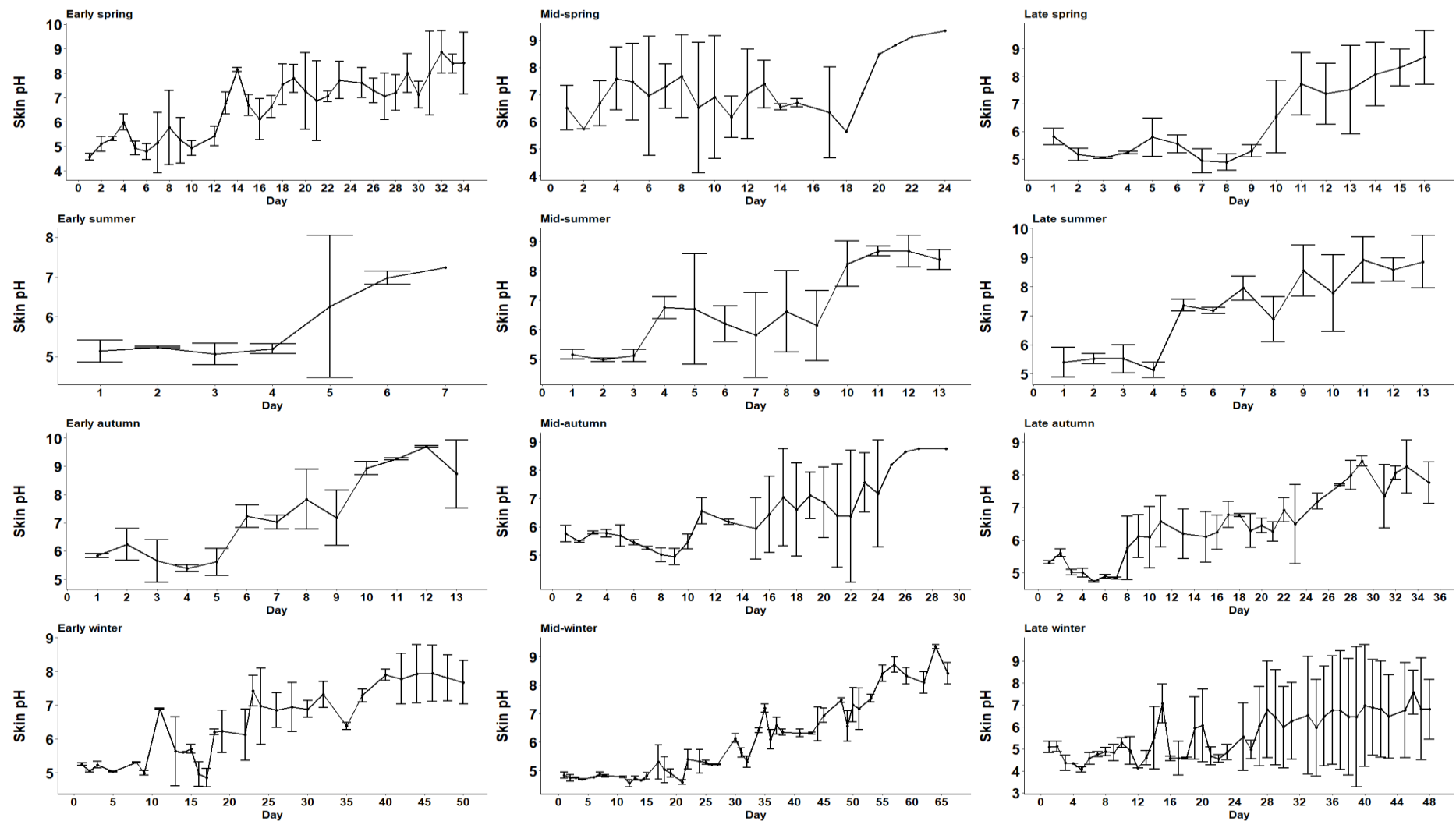


Figure 6.3: Daily mean carcass skin pH in each month across the spring, summer, autumn, and winter seasonal periods. Error bars represent standard deviations where $n = 2$.

6.1.4. Larval mass pH

Apart from the early and mid-winter trials where the larval masses were not large and compact, most of the larval masses across the seasonal trials were compact enough to receive a pH probe on different days, largely between Days 3 and 10 (Table 6.6). Generally, the larval masses pH increased as decomposition progressed and were mostly above the neutral pH mark (Figure 6.4).

Table 6.6: Summary of Days in which larval mass(es) were compact enough to receive a pH probe in each monthly neonate pig decomposition trial across the spring, summer, autumn and winter seasons.

Trial	Days when larval mass pH was taken			
	Spring	Summer	Autumn	Winter
Early	Days 12 – 19	Days 3 – 6	Days 3 – 6	-
Mid	Days 4 – 15	Days 4 – 11	Days 6 – 11	-
Late	Days 5, 12 – 15	Days 3 – 10	Days 10 – 11, 17 – 19	Days 9 – 20, 33 – 41

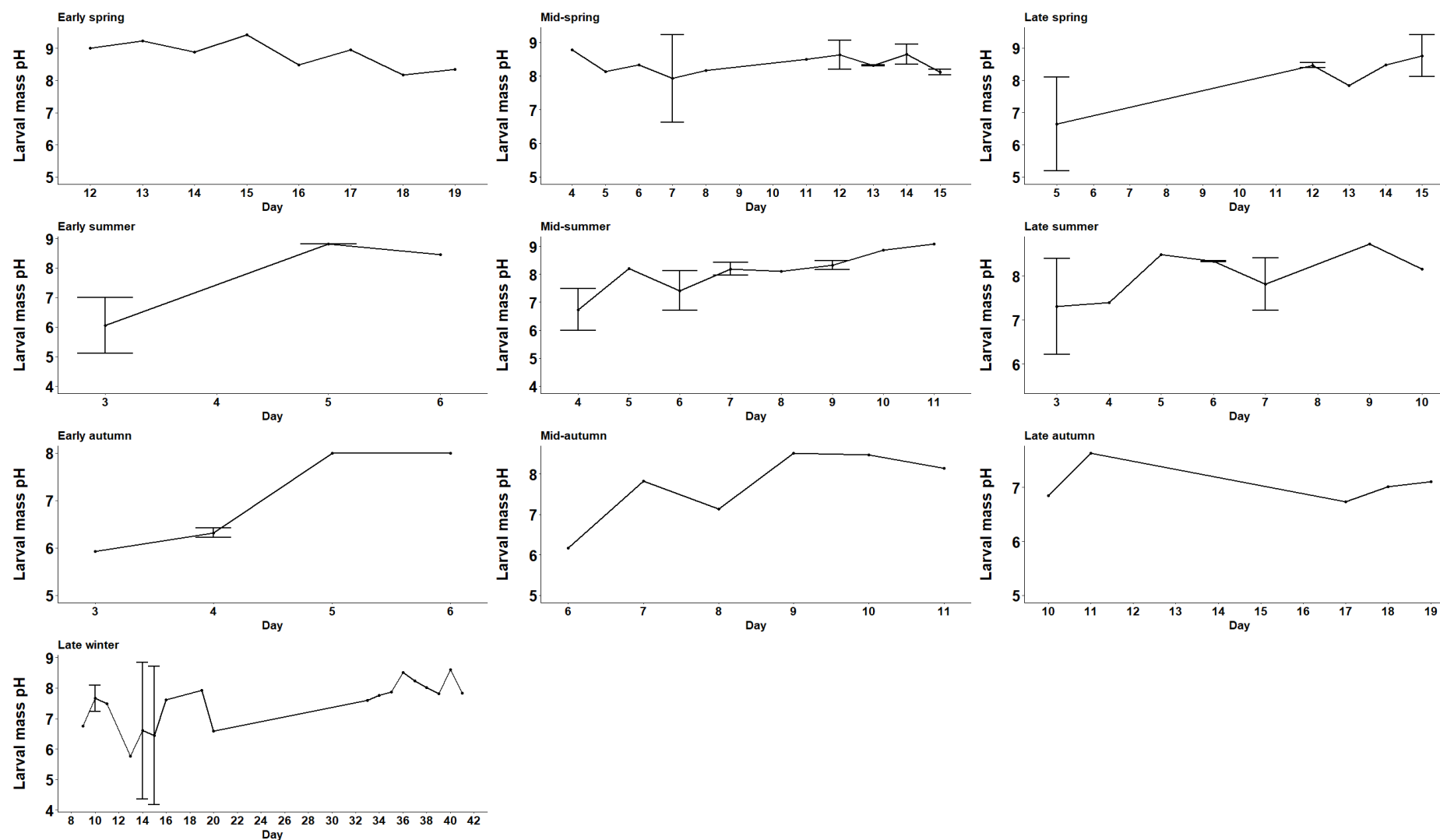


Figure 6.4: Daily mean larval mass(es) pH in each month across the spring, summer, autumn, and winter seasonal periods. Error bars represent standard deviations where $n \geq 2$.

6.1.5. Modelling $\Delta\text{pH}/\Delta\text{EC}$ with ADD, ambient relative humidity and rainfall

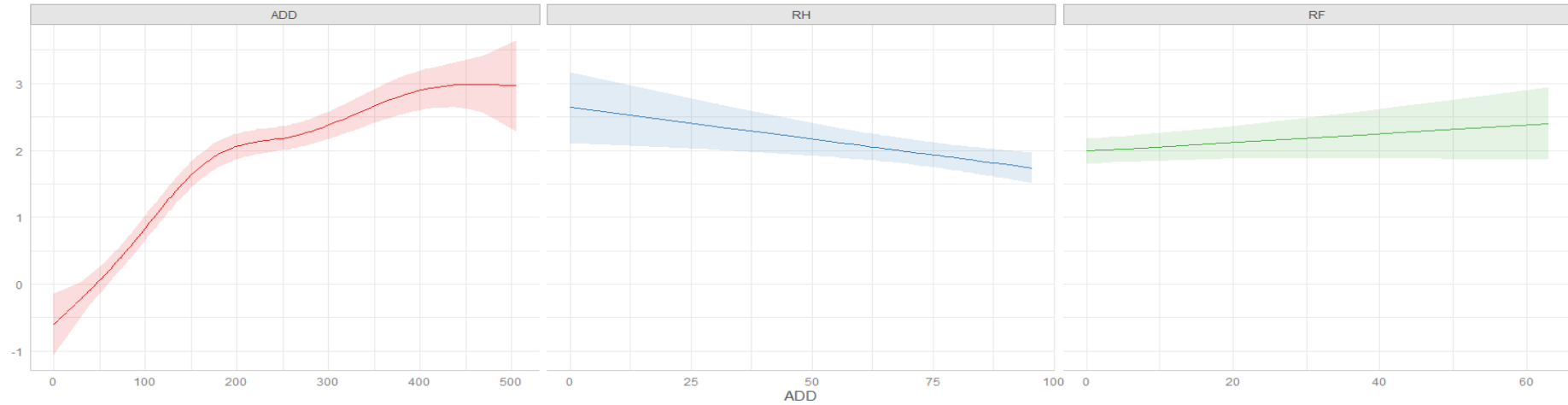
Warm seasonal period

The GAM output of the dependent variable, ΔpH and independent variables, ADD, ambient relative humidity, and rainfall in the warm seasonal period was curvilinear (Figure 6.5). The output from the GAM analysis showed that ADD ($p < 0.0001$) and ambient relative humidity ($p = 0.005$) had a significant effect on the changes in soil pH. In contrast, rainfall was found to have no significant effect on the changes in soil pH ($p = 0.11$). Furthermore, the GAM model had an adjusted R^2 value of 0.68 indicating that 68% of the variability in soil pH as decomposition progresses could be attributed to the ADD and ambient relative humidity. Similarly, a curvilinear relationship was produced following the modelling of ΔEC with ADD, ambient relative humidity, and rainfall within the warm seasonal period (Figure 6.6). Here, both ADD ($p < 0.0001$), and ambient relative humidity ($p < 0.0001$) had a significant effect on the changes in soil electrical conductivity, while the effect of rainfall was not significant ($p = 0.44$). An adjusted R^2 value of 0.43 was produced, implying that 43% of the variability in soil electrical conductivity was linked to the effect of ADD and ambient relative humidity.

Cold seasonal period

In the cold seasonal period, the GAM output of ΔpH with ADD, ambient relative humidity, and rainfall was curvilinear (Figure 6.5). Only ADD was found to have a significant ($p < 0.0001$) impact on the changes in soil pH, while ambient relative humidity ($p = 0.2$) and rainfall ($p = 0.73$) exerted no significant impact. The model produced an adjusted R^2 value of 0.47. This shows that ADD moderately (47%) accounts for the variation in soil pH. Likewise, ADD was found to have a significant ($p < 0.0001$) impact on the electrical conductivity of the soil, when compared to ambient relative humidity ($p = 0.12$) and rainfall ($p = 0.37$) both of which had no significant influence (Figure 6.6). In spite of this, only 22% (adjusted R^2 value = 0.22) of the variability in soil electrical conductivity could be accounted for by ADD.

Relationship between ΔpH , ADD, RH, and RF in the Warm seasonal period



Relationship between ΔpH , ADD, RH, and RF in the Cold seasonal period

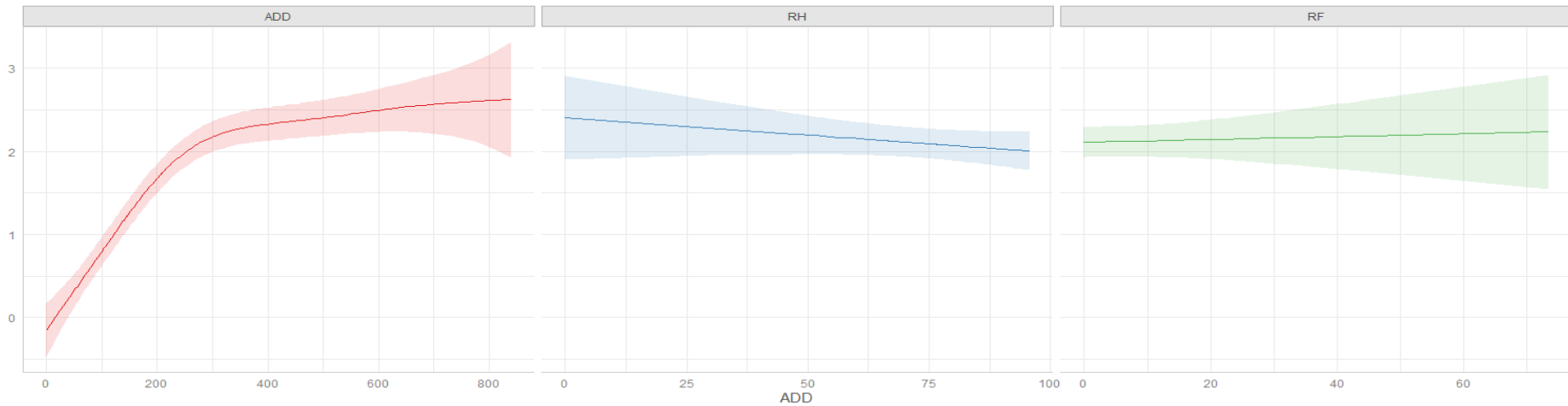


Figure 6.5: Relationship between ΔpH and ADD (first panel), ambient relative humidity (second panel), and rainfall (third panel) in the warm (top plot) and cold (bottom plot) seasonal periods based on GAM models. Shading indicates standard error.

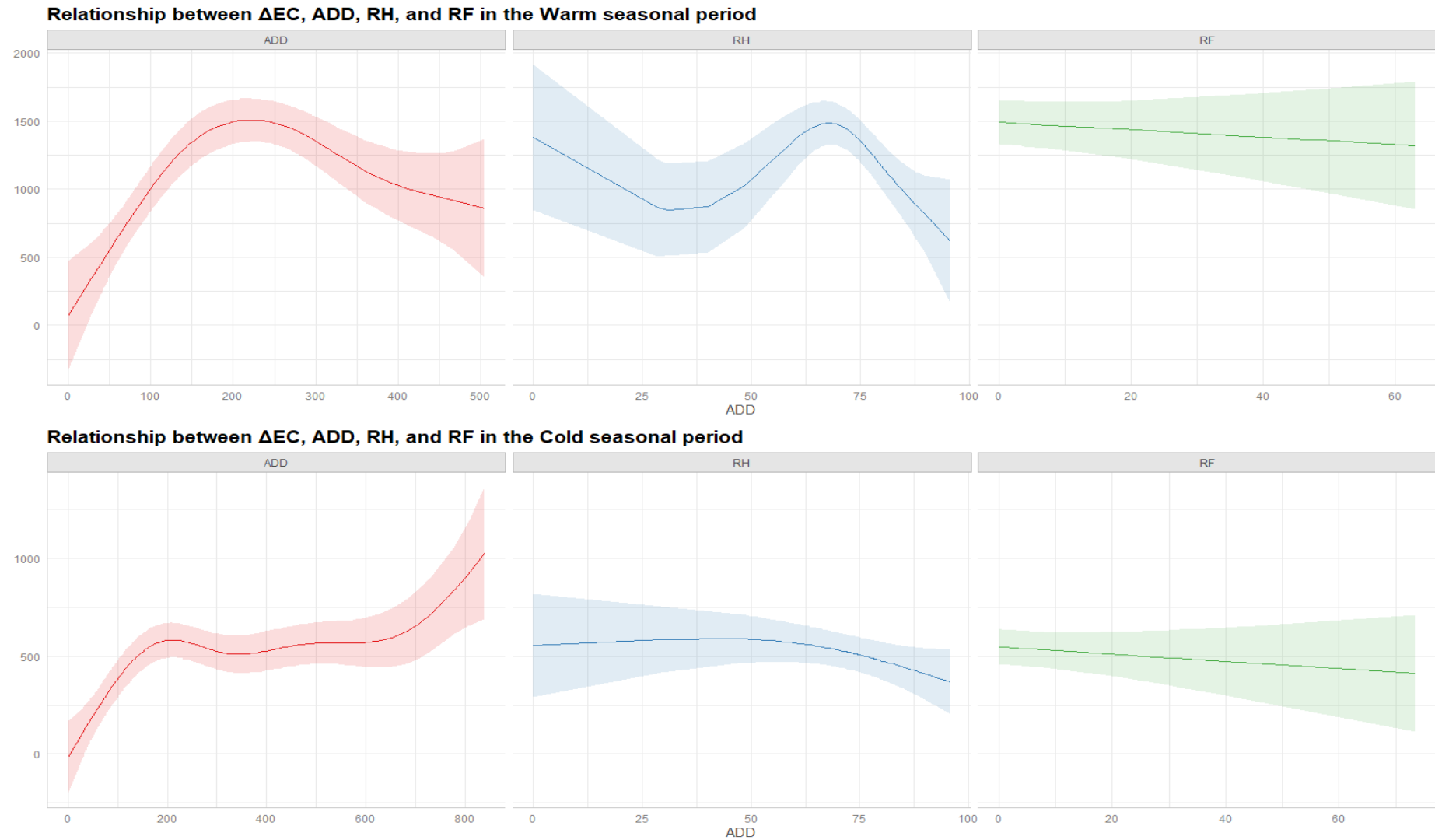


Figure 6.6: Relationship between ΔEC and ADD (first panel), ambient relative humidity (second panel) and rainfall (third panel) in the warm (top plot) and cold (bottom plot) seasonal period based on GAM models. Shading indicates standard error.

6.1.6. Modelling carcass skin pH with ADD, ambient relative humidity and rainfall

The carcass skin pH data generated in the warm (from December to May) and cold (from June to November) seasonal periods were respectively pooled together to create two distinct datasets for the warm and cold seasons as above. Similarly, ADD, ambient relative humidity and rainfall data generated during these periods were pooled together. Using GAMs, the pooled warm and cold seasons' carcass skin pH data were respectively modelled as a function of the pooled ADD, ambient relative humidity and rainfall datasets within the same periods.

In the warm seasonal period, the result from the GAM analysis revealed that the changes in carcass skin pH were significantly influenced by ADD ($p < 0.0001$) and ambient relative humidity ($p = 0.005$) (Figure 6.7). However, rainfall had no significant ($p = 0.12$) impact on carcass skin pH. In addition, the GAM model produced an adjusted R^2 value of 0.48 suggesting that the variability in the changes in carcass skin pH as cadaver decomposition progresses could be moderately (48%) attributed to ADD and ambient relative humidity. In the cold seasonal period, only ADD ($p < 0.0001$) exerted a significant influence on the carcass skin pH (Figure 6.7). However, ambient relative humidity ($p = 0.38$) and rainfall ($p = 0.12$) showed no significant impact on the carcass skin pH (Figure 6.7). Here, an adjusted R^2 value of 0.34 was produced and this suggests that 34% of the variability in carcass skin pH may be attributed to ADD.

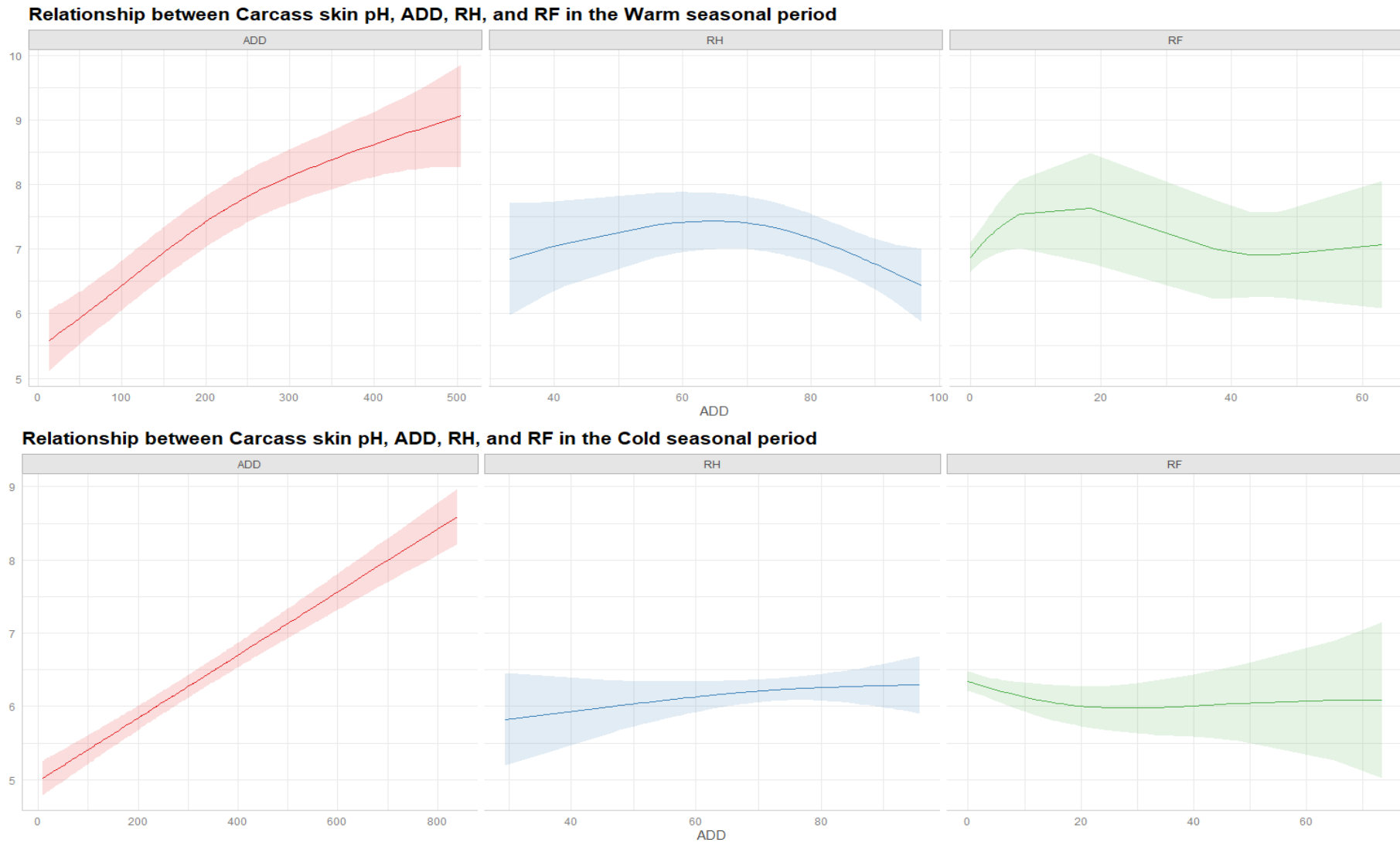


Figure 6.7: Relationship between carcass skin pH and ADD (first panel), ambient relative humidity (second panel) and rainfall (third panel) in the warm (top plot) and cold (bottom plot) seasonal period based on GAM models. Shading indicates standard error.

6.2. Adult pig summer trial

6.2.1. Soil pH

In this trial, the pH of the carcass soil (7.4 ± 1.2) was significantly higher ($p = 0.03$) than that of the control soil (5.9 ± 0.2). There was a significant ($F_{3,4} = 7.76$; $p = 0.04$) difference in the pH of the carcass soil with respect to the decomposition stage of the carcass. However, pairwise comparisons did not reveal any statistically significant ($p > 0.05$) differences across the fresh (5.7), early decomposition (6.3 ± 0.4), advanced decomposition (8.3) and skeletonization (8.2 ± 0.7) stages. The control soil pH ranged between 5.65 and 6.22 . Carcass soil pH began to increase steadily on Day 4, exceeding the neutral pH mark on Day 6 with a peak value of 8.7 on Day 11 (Figure 6.8).

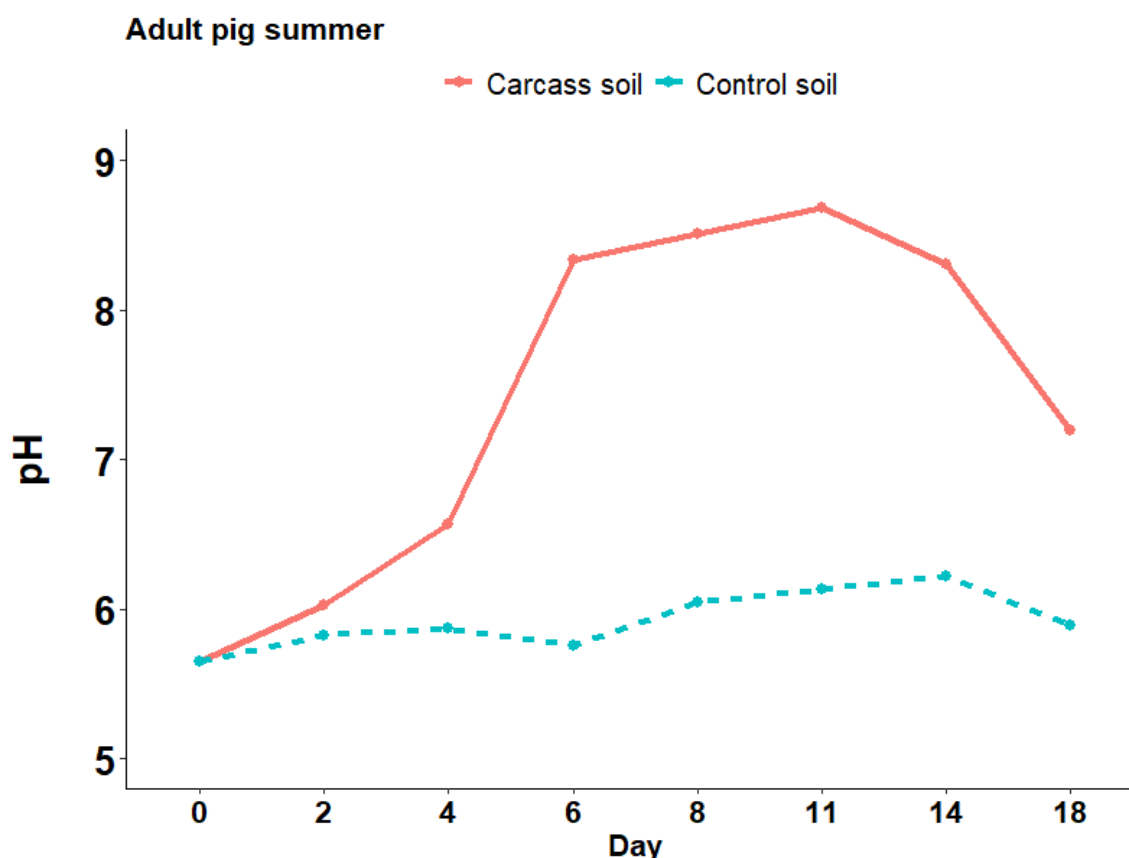


Figure 6.8: Daily pH of carcass (red solid lines) and control (blue broken lines) soils in the adult pig summer trial.

6.2.2. Soil electrical conductivity

The electrical conductivity of the soil underneath the pig carcass (1262.5 ± 1064) was significantly ($p = 0.005$) higher than that of the control soil (121.3 ± 27.8). Carcass soil electrical conductivity was significantly ($F_{3,4} = 10.07$; $p = 0.02$) impacted by the decomposition stage of the carcass. Pairwise comparison revealed no statistically significant ($p > 0.05$) differences in the electrical conductivity of the carcass soil across the fresh (155.1), early decomposition (139 ± 18.3) and skeletonization (1710.2 ± 555.7) stages. However, the electrical conductivity of the carcass soil during the advanced decomposition stage (2826) was significantly ($p < 0.05$) higher than what was observed during the early decomposition stage but not the other stages of decomposition. In the carcass soil, the electrical conductivity exceeded the maximum value recorded for the control soil on Day 6 (2826), and then declined to a value of 896.6 on Day 8 (Figure 6.9). However, from Day 11 till the termination of the trial, carcass soil electrical conductivity ranged between 1814 to 2097 $\mu\text{S}/\text{cm}$ (Figure 6.9).

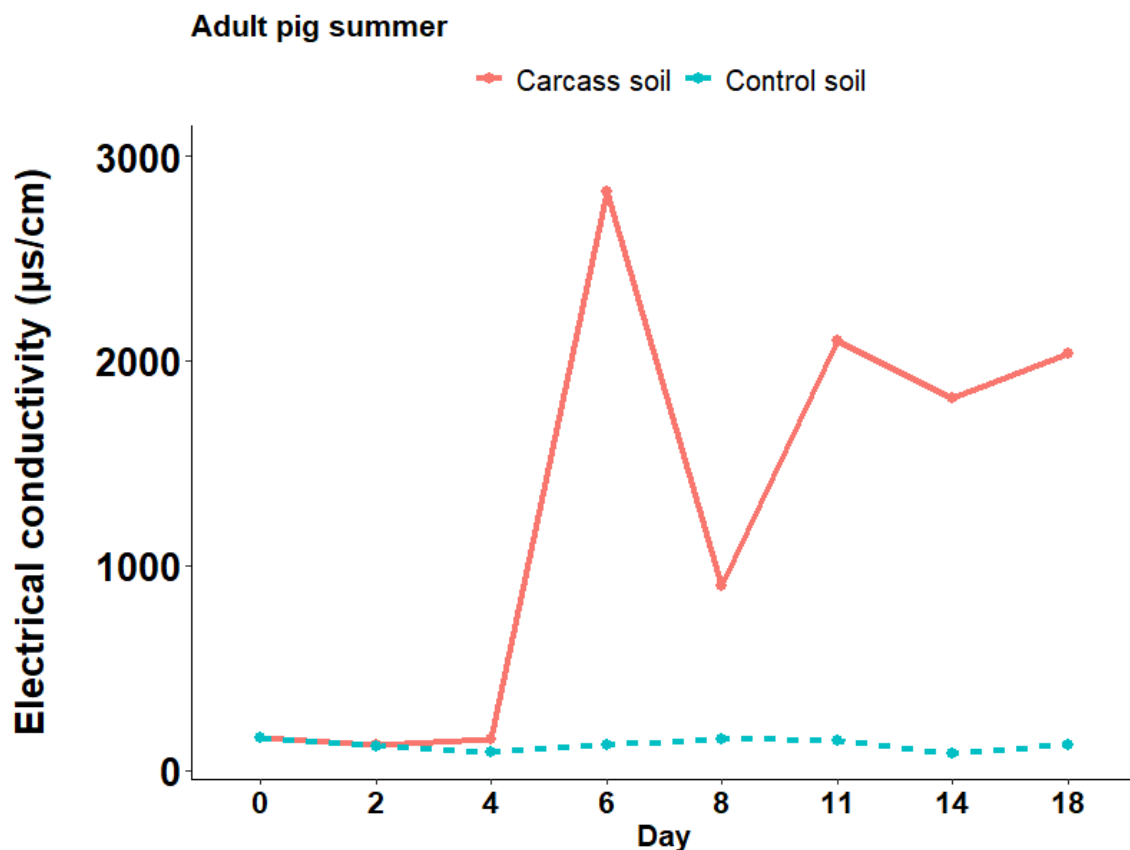


Figure 6.9: Daily electrical conductivity of carcass (red solid lines) and control (blue broken lines) soils in the adult pig summer trial.

6.2.3. Carcass skin pH

In this trial, the pH of the carcass skin did not differ significantly ($F_{2,9} = 0.85$; $p = 0.46$) across the early decomposition (6.8 ± 1.1), advanced decomposition (7.3 ± 1) and skeletonization (7.5 ± 0.6) stages of the carcass. The carcass skin pH was initially acidic specifically between Days 1 and 2 (Figure 6.10). However, from Days 3 to 6, carcass skin pH exceeded the neutral pH mark and was mainly alkaline. On Day 7, carcass skin pH returned to being acidic, but this was short-lived as there was an acid to alkaline shift in carcass skin pH the following day (i.e., Day 8, pH = 7.23), with peak skin pH of 8.49 recorded on Day 9 (Figure 6.10). Till the end of the experimental trial, the carcass skin pH was largely alkaline.

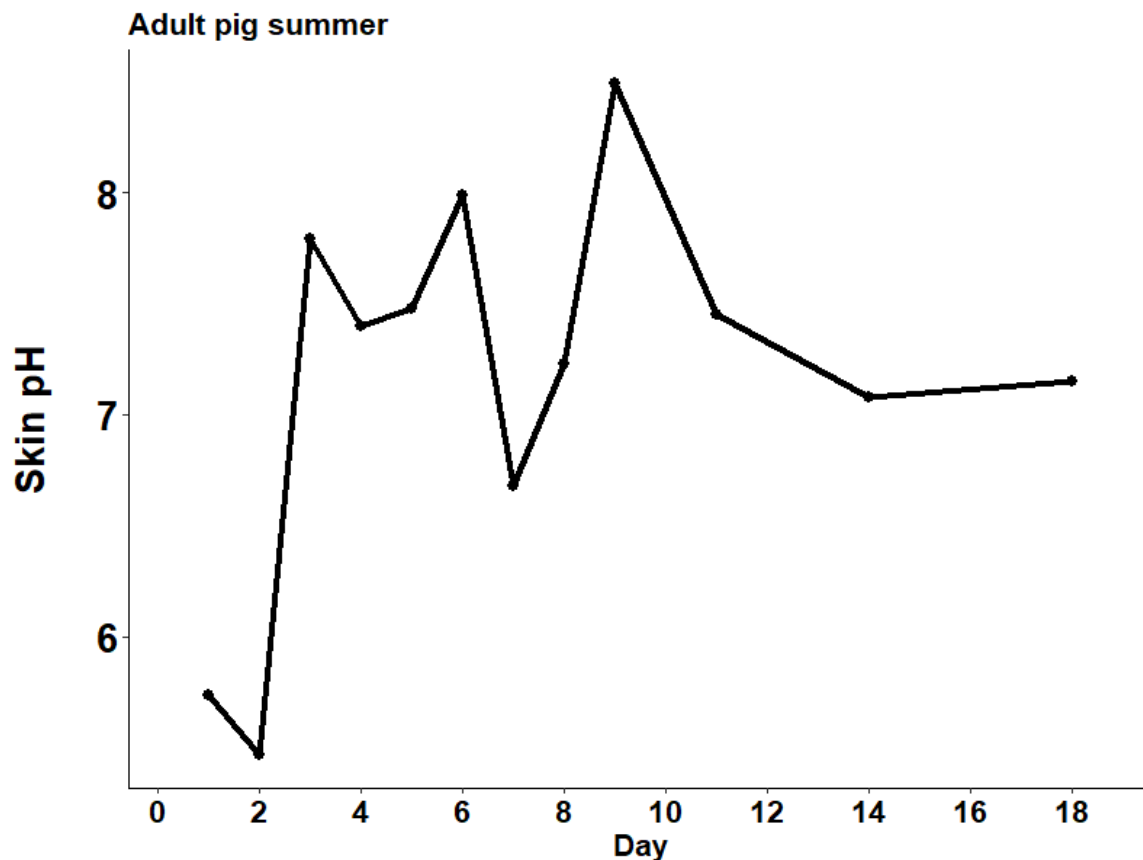


Figure 6.10: Daily carcass skin pH in the adult pig summer trial.

6.2.4. Larval mass pH

The larval masses on the carcass were compact enough to receive a probe from Days 4 to 7. Here, larval masses were largely an alkaline environment with pH ranging from 7.04 to 8.08 (Figure 6.11).

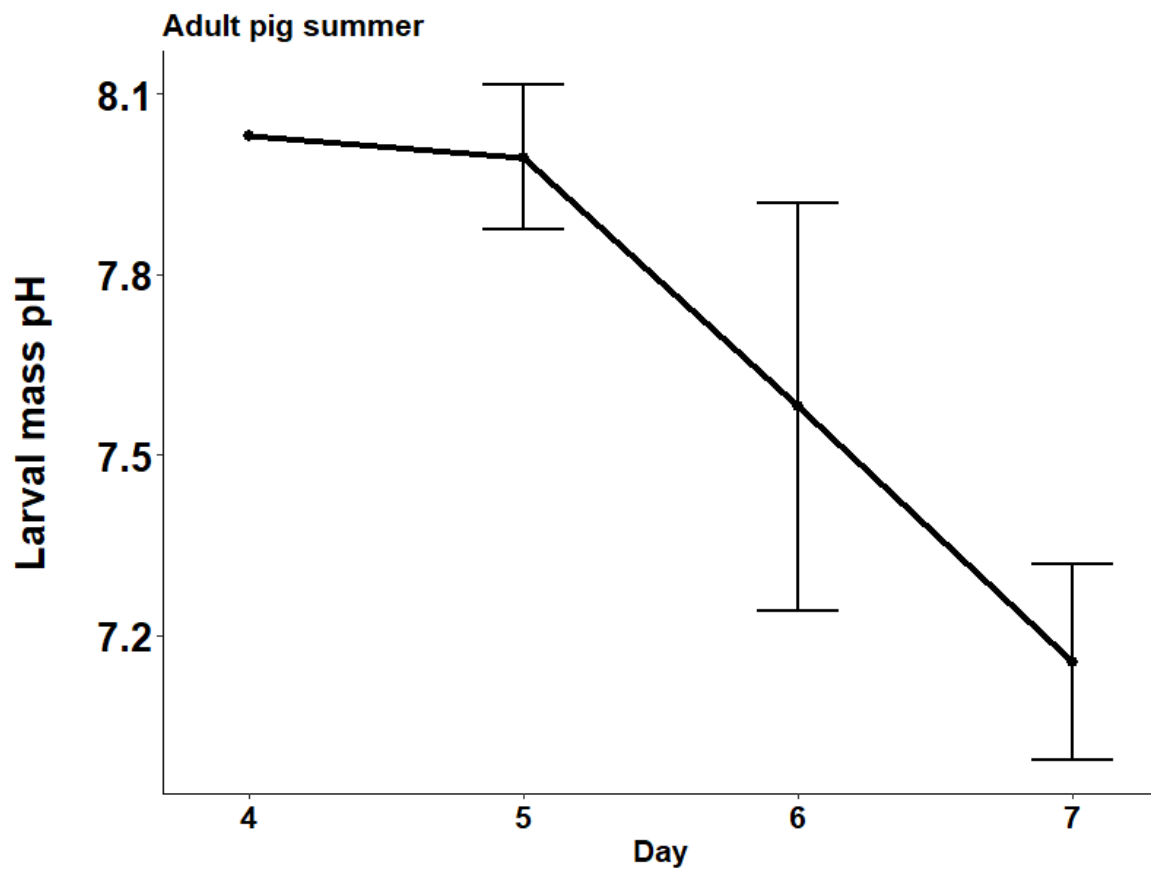


Figure 6.11: Daily mean larval mass(es) pH in the adult pig summer trial. Error bars represent standard deviations where $n \geq 2$.

6.2.5. Influence of carcass size on carcass soil pH and electrical conductivity in the summer season

Adult pig trial vs early summer neonate pig trial

Within this period, the pH (t-test = 1.7; DF = 10.18; $p = 0.12$) and electrical conductivity ($p = 0.46$) of the carcass soil did not differ significantly with respect to the size of the carcass (Figures 6.12A & 6.12D). Similarly, there were no significant differences in the pH (t-test = 0.38; DF = 19.4; $p = 0.7$) and electrical conductivity ($p = 0.07$) of the surrounding control soil of the adult pig and neonate pig carcasses (Figures E1A & E1D).

Adult pig trial vs mid-summer neonate pig trial

Here, there was no significant difference in the pH ($p = 0.5$) and electrical conductivity (t-test = -0.94; DF = 9.86; $p = 0.37$) of the carcass soil between the neonate and adult pig carcasses (Figures 6.12B & 6.12E). In contrast, the pH ($p < 0.001$) and electrical conductivity (t-test = -4.79; DF = 9.1; $p < 0.001$) of the control soil surrounding the neonate pig carcasses were significantly higher than those of the adult pig carcass (Figures E1B & E1E).

Adult pig trial vs late summer neonate pig trial

Within these two periods, carcass soil pH ($p = 0.37$) and electrical conductivity ($p = 0.43$) did not vary significantly between the neonate and adult pig carcasses (Figures 6.12C & 6.12F). On the contrary, the pH (t-test = -8.02; DF = 14.8; $p < 0.0001$) and electrical conductivity ($p = 0.007$) of the control soil surrounding the neonate pig carcasses were significantly higher than those of the adult pig carcass (Figures E1C & E1F).

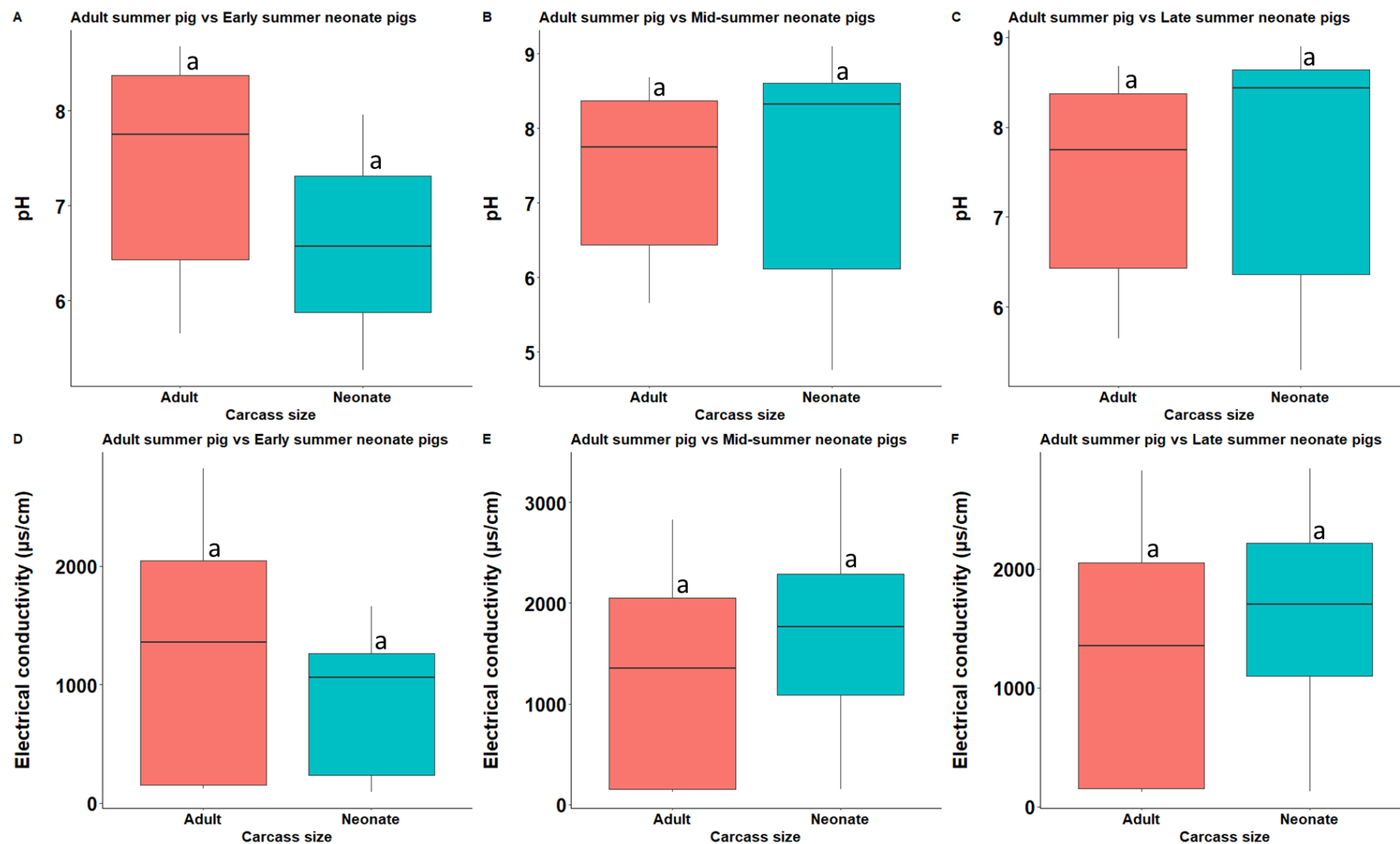


Figure 6.12: Box plots comparing the carcass soil pH (A – C) and electrical conductivity (D – E) of the adult summer pig carcass and neonate pig early, mid-, and late summer carcasses. Boxes capped with different letters are significantly different after Welch Two Sample t-test or Wilcoxon signed-rank test ($p < 0.05$).

6.2.6. Influence of carcass size on carcass skin pH in the summer season

The pH of the adult pig carcass skin in the summer season was significantly ($p = 0.003$) higher than that of the neonate pig carcasses during the early summer seasonal period (Figure 6.13A). In contrast, there were no significant differences in the adult pig carcass skin pH when compared to those of the neonate pig carcasses in the mid- ($p = 0.38$) and late summer ($p = 0.82$) seasonal periods (Figures 6.13B & 6.13C).

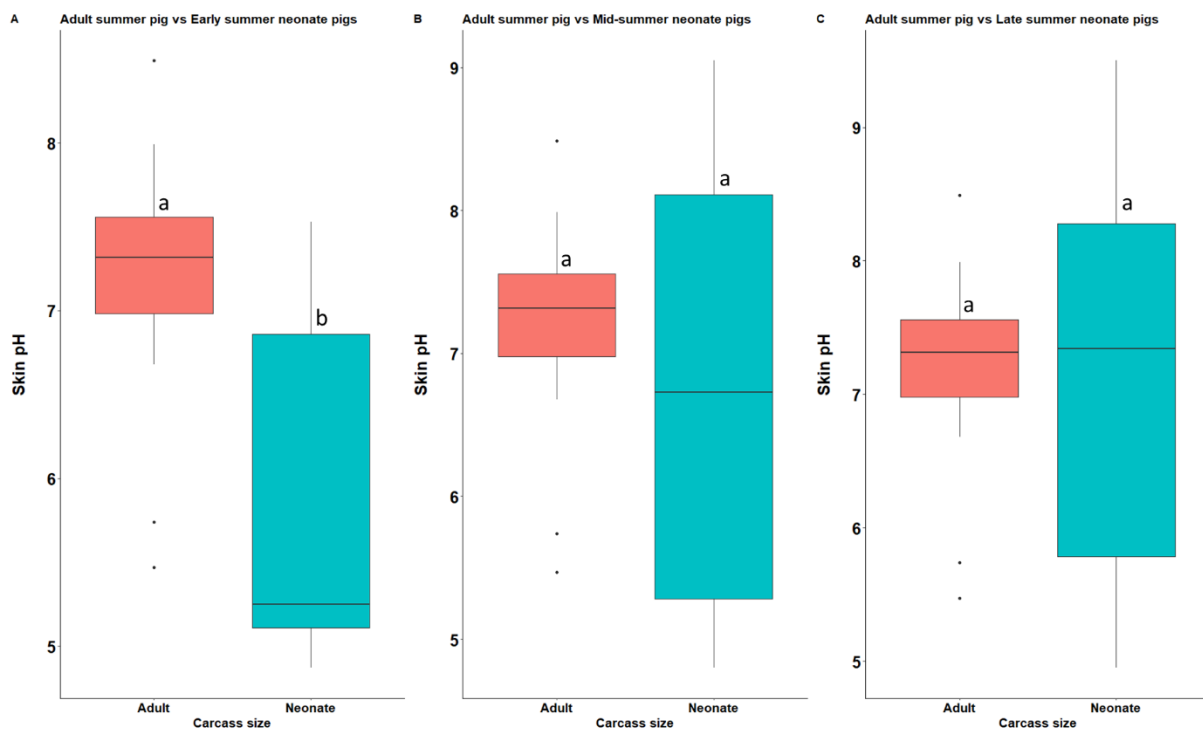


Figure 6.13: Box plots comparing the skin pH (A – C) of the adult summer pig carcass and neonate pig early, mid-, and late summer carcasses. Boxes capped with different letters are significantly different after Wilcoxon signed-rank test ($p < 0.05$).

6.3. Adult pig winter trial

6.3.1. Soil pH

In this trial, the pH of the soil underneath the pig carcass (7.8 ± 1.3) was significantly ($p < 0.0001$) higher than that of the control soil (5.7 ± 0.3). In addition, the changes in carcass soil pH were significantly (Kruskal-Wallis $\chi^2 = 12.19$; $DF = 3$; $p = 0.006$) influenced by the decomposition stage of the carcass. The pH of the carcass soil during the advanced decomposition stage (8.4 ± 0.7) was significantly ($p < 0.05$) higher than what was recorded while the carcass was still fresh (5.9 ± 0.2). However, there were no significant ($p > 0.05$) differences in the carcass soil pH across the fresh, early decomposition (7.9 ± 1.5) and skeletonization (7.0 ± 1.2) stages. Similarly, no significant ($p > 0.05$) difference was observed in the pH of the carcass soil across the early, advanced decomposition and skeletonization stages. Here, the control soil pH ranged between 5.1 and 7.0 (Figure 6.14). Carcass soil pH exceeded the neutral pH mark on Day 6 with a peak value of 9.2 on Day 90 (Figure 6.14).

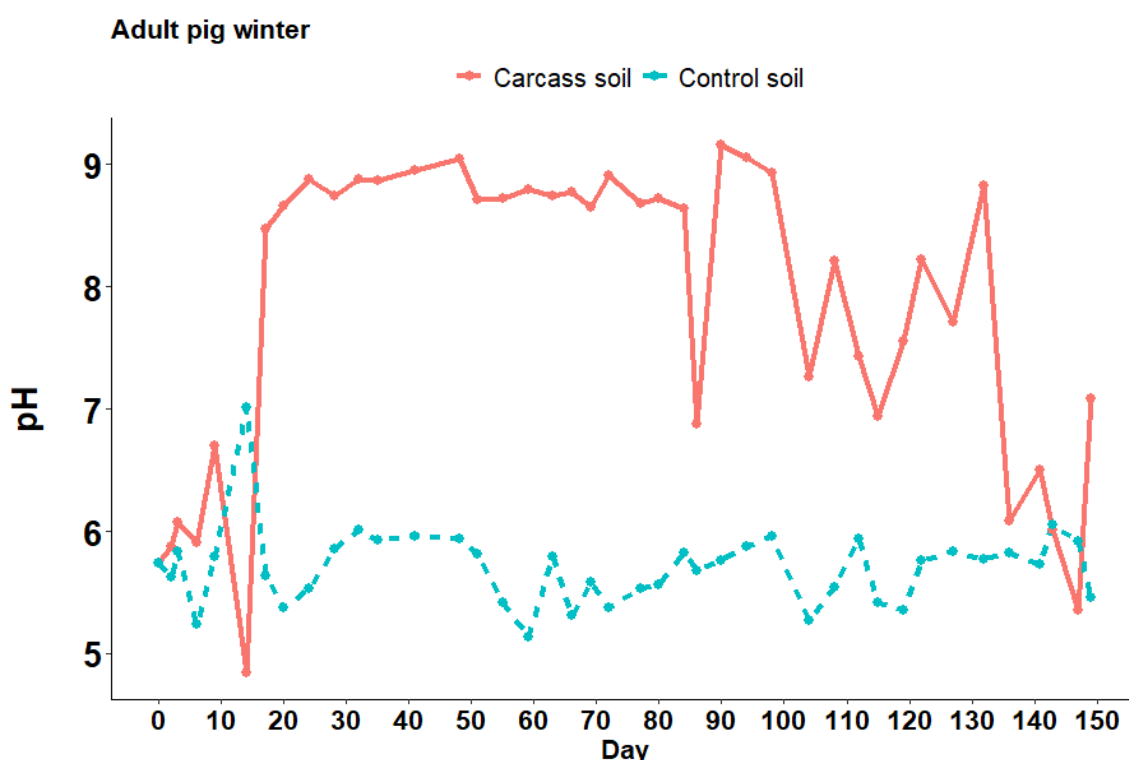


Figure 6.14: Daily pH of carcass (red solid lines) and control (blue broken lines) soils in the adult pig winter trial.

6.3.2. Soil electrical conductivity

The electrical conductivity of the carcass-associated soil (981.2 ± 589.1) was significantly ($p < 0.0001$) higher than the control soil (121.1 ± 25). In relation to the decomposition stage of the carcass, the electrical conductivity of the carcass soil was observed to vary significantly (Kruskal-Wallis $\chi^2 = 14.71$; $DF = 3$; $p = 0.002$). During the skeletonization stage (1494.1 ± 641.4), the electrical conductivity of the carcass soil was significantly ($p < 0.05$) higher than the recorded values while the carcass was in the fresh (105.3 ± 9) and early decomposition (699.2 ± 509.2) stages. However, no significant ($p > 0.05$) difference was observed between the advanced decomposition (1048.5 ± 421.5) and skeletonization stages. Similarly, carcass soil electrical conductivity did not differ significantly ($p > 0.05$) between the early decomposition and skeletonization stages. The electrical conductivity of the carcass soil peaked on Day 132 (2912). Apart from Days 0 – 14 and 132, the carcass soil electrical conductivity ranged between 338.5 to 1760 $\mu\text{S}/\text{cm}$ (Figure 6.15).

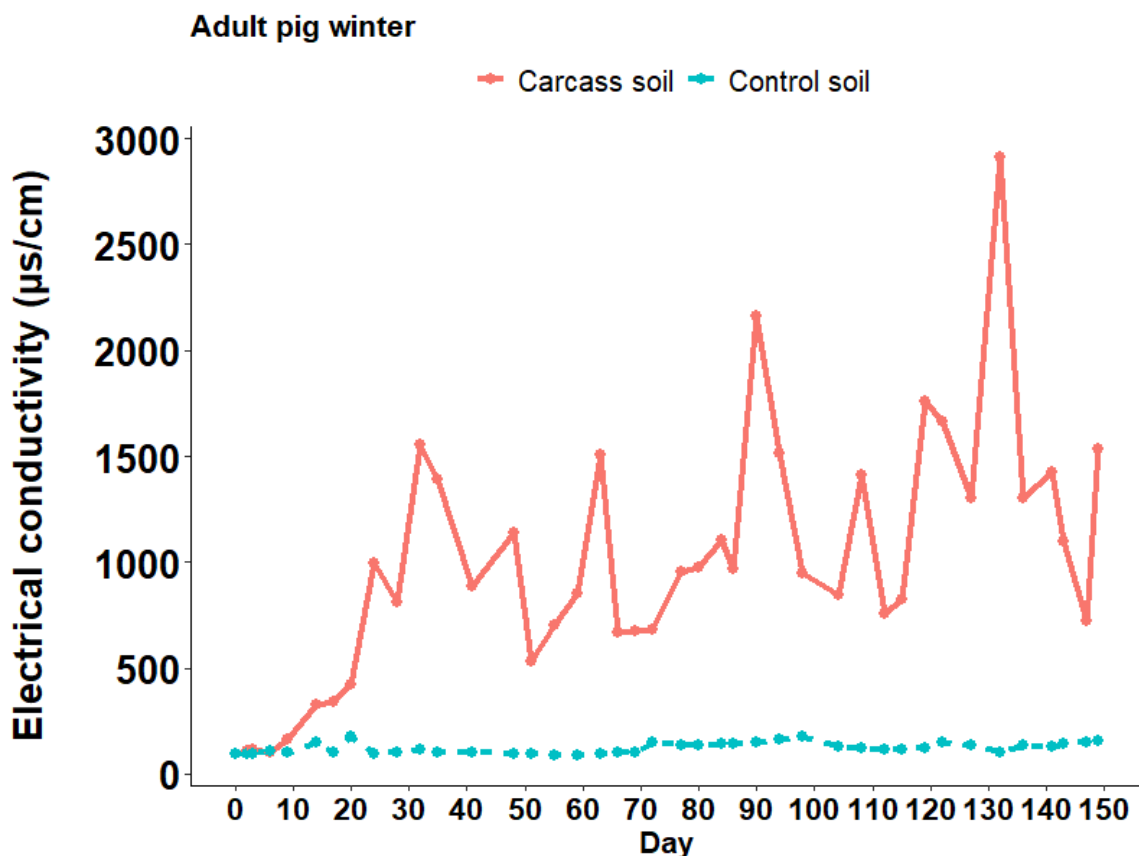


Figure 6.15: Daily electrical conductivity of carcass (red solid lines) and control (blue broken lines) soils in the adult pig winter trial.

6.3.3. Carcass skin pH

In this trial, the pH of the carcass skin varied significantly (Kruskal-Wallis $\chi^2 = 18.94$; DF = 3; $p < 0.001$) in relation to the decomposition stages. Significantly ($p < 0.05$) higher skin pH was observed during the advanced decomposition (7.7 ± 0.3) stage of the pig carcass when compared to the fresh (6.3 ± 0.6) and early decomposition (7.3 ± 0.6) stages. However, there were no significant ($p > 0.05$) differences in the pH of the carcass skin across the fresh, early decomposition and skeletonization (7.4 ± 0.3) stages. Similarly, carcass skin pH did not differ significantly ($p > 0.05$) between the advanced decomposition and skeletonization stages. There was a considerable fluctuation in carcass skin pH in this trial. The carcass skin was an acidic environment at the start of the experimental trial until Day 4 where a shift to alkalinity was noted (Figure 6.16). However, between Day 6 until the end of the trial, the carcass skin pH continuously fluctuated between acidity and alkalinity. Despite this, the carcass skin was majorly an alkaline environment especially during the late stages of decomposition (Figure 6.16).

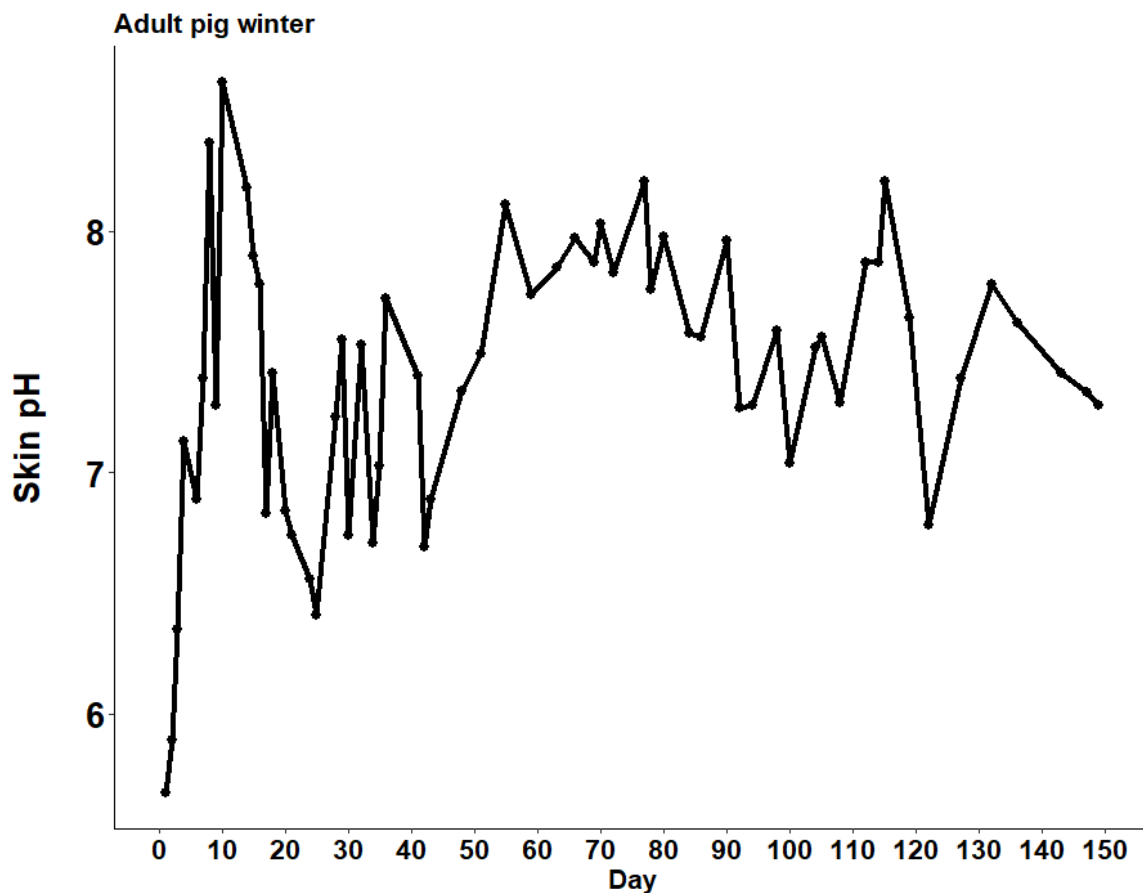


Figure 6.16: Daily carcass skin pH in the adult pig winter trial.

6.3.4. Larval mass(es) pH

The larval masses on the carcass were compact enough to receive a probe from Days 30 to 55 (Figure 6.17). Here, larval masses were majorly an alkaline environment with pH ranging from 8.3 to 9.5 (Figure 6.17).

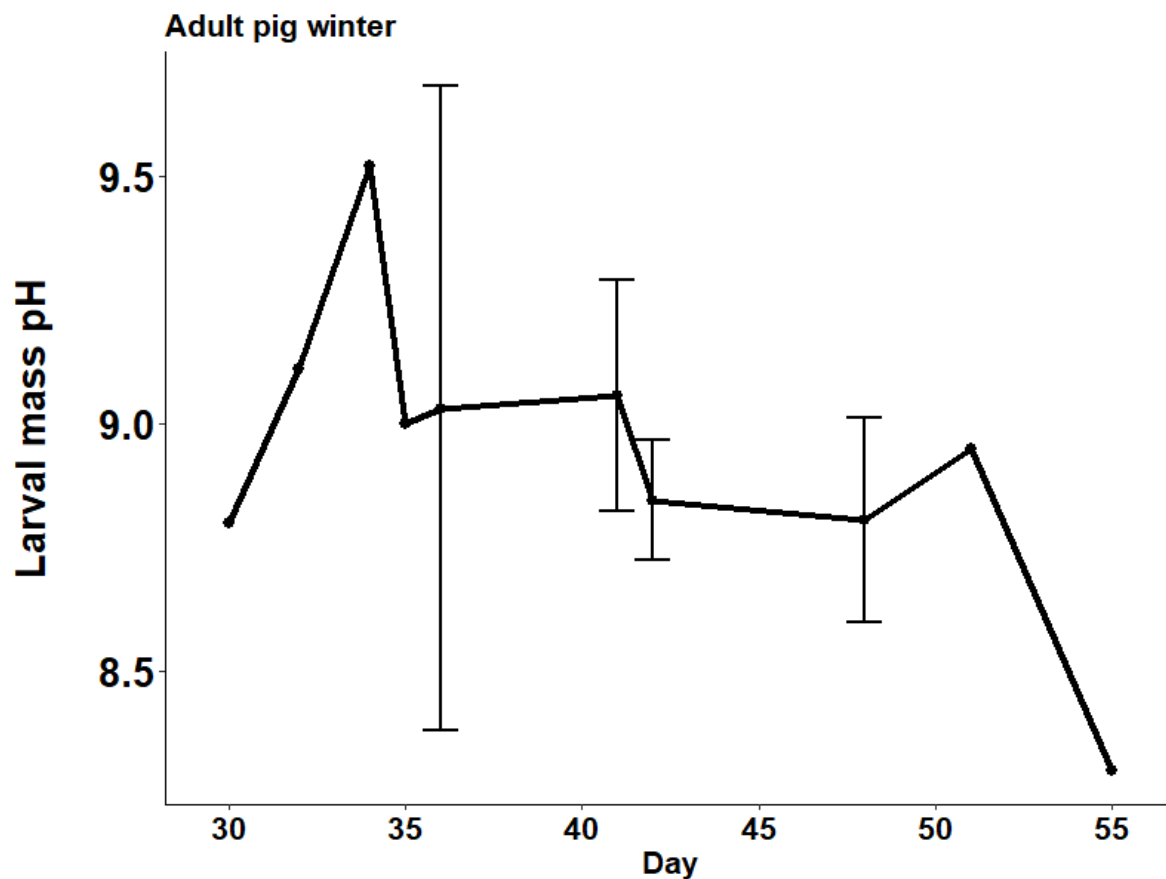


Figure 6.17: Daily mean larval mass(es) pH in the adult pig winter trial. Error bars represent standard deviations where $n \geq 2$.

6.3.5. Influence of carcass size on carcass soil pH and electrical conductivity in the winter season

Adult pig trial vs early winter neonate pig trial

Within this period, the pH of the soil underneath the neonate and adult pig carcasses did not differ significantly ($p = 0.69$) (Figure 6.18A). However, the electrical conductivity of the soil underneath the adult pig carcass was significantly ($p < 0.0001$) higher than that of the neonate pig carcasses (Figure 6.18D). With respect to the control soil, the adult pig carcass control soil pH did not differ significantly ($p = 0.26$) from that of the neonate pig carcasses, however, significantly ($p = 0.02$) higher electrical conductivity was observed in the neonate pig carcasses control soil when compared to the adult pig carcass (Figures E2A & E2D).

Adult pig trial vs mid-winter neonate pig trial

The pH of the soil underneath the neonate and adult pig carcasses were not significantly ($p = 0.98$) different (Figure 6.18B). In contrast, the soil associated with the adult pig carcass had significantly ($p < 0.0001$) higher electrical conductivity when compared to the neonate pig carcass soil (Figure 6.18E). Here, there were no significant differences in the pH ($p = 0.22$) and electrical conductivity ($p = 0.06$) of the control soil surrounding the adult and neonate pig carcasses (Figures E2B & E2E).

Adult pig trial vs late winter neonate pig trial

Within these periods, both the pH ($p < 0.0001$) and electrical conductivity ($p < 0.0001$) of the soil underneath the adult pig carcass were significantly higher than those of the neonate pig carcasses (Figures 6.18C & 6.18F). In contrast, the pH (t-test = -6.19; DF = 91.86; $p < 0.0001$) and electrical conductivity ($p < 0.001$) of the neonate pig carcasses control soil were significantly higher than those of the adult pig carcass (Figures E2C & E2F).

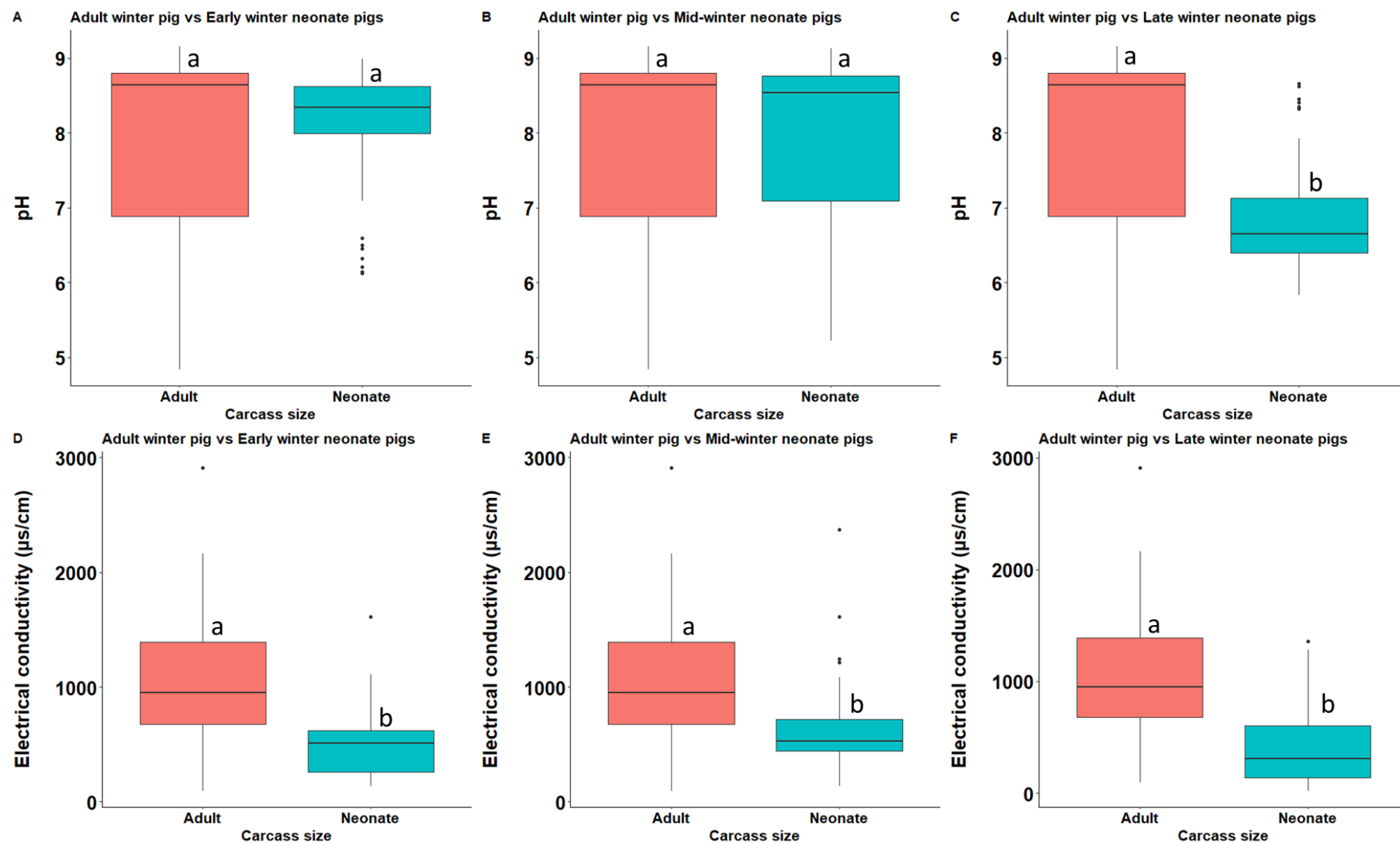


Figure 6.18: Box plots comparing the carcass soil pH (A – C) and electrical conductivity (D – E) of the adult winter pig carcass and neonate pig early, mid-, and late winter carcasses. Boxes capped with different letters are significantly different after Wilcoxon signed-rank test ($p < 0.05$).

6.3.6. Influence of carcass size on carcass skin pH in the winter season

The pH of the adult pig carcass skin in the winter season was significantly higher than that of the neonate pig carcasses during the early ($p < 0.0001$), mid- ($p < 0.0001$) and late winter ($p < 0.0001$) seasonal periods (Figures 6.19A – C).

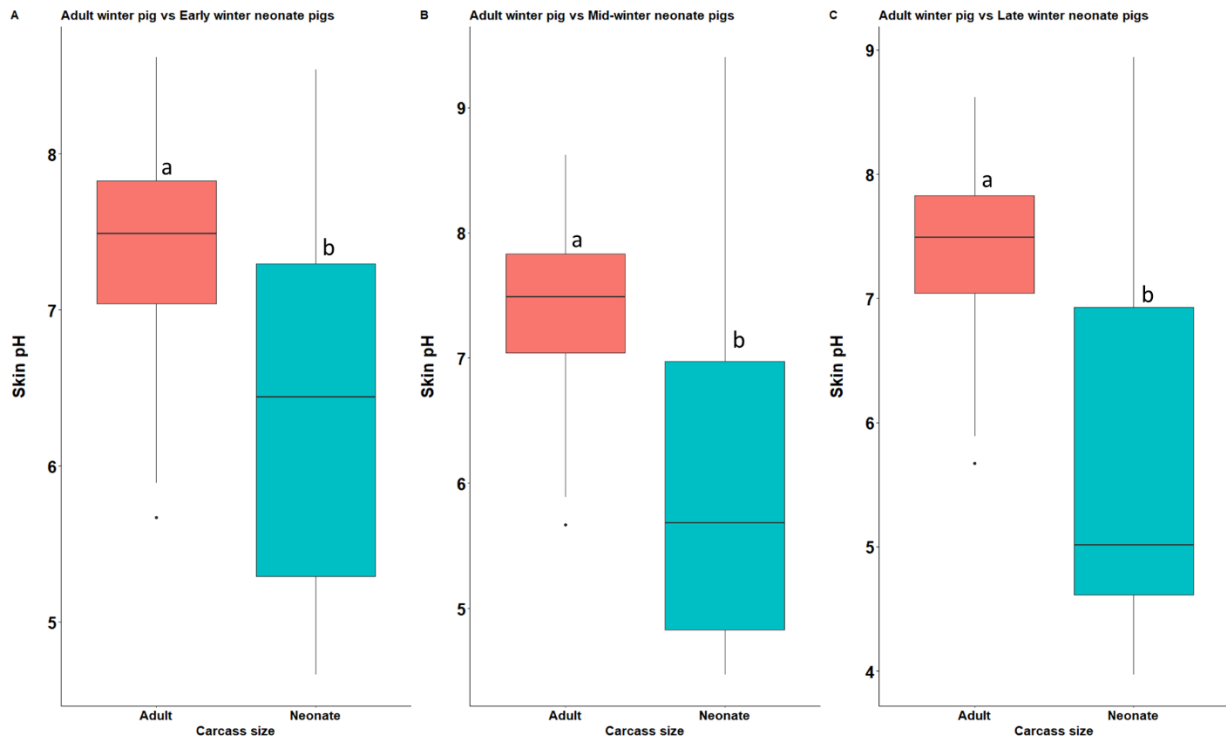


Figure 6.19: Box plots comparing the skin pH (A – C) of the adult winter pig carcass and neonate pig early, mid-, and late winter carcasses. Boxes capped with different letters are significantly different after Wilcoxon signed-rank test ($p < 0.05$).

6.4. Discussion

The impact of decomposing neonate and adult pig carcasses on the pH and electrical conductivity of underlying soils in different seasons was investigated. Despite the inconsistencies in the reports from different parts of the world in relation to the response of some selected soil chemical parameters (e.g., pH) (see Singh *et al.*, 2023) to decomposing vertebrate remains, no published studies, to the best of the author's knowledge, exist on the impact of cadaver decomposition on soil pH and electrical conductivity across multiple seasons in Africa. Therefore, this study is the first to empirically investigate and quantify the effects of carcass decomposition on soil chemistry in Africa.

In each of the monthly neonate pig trials across all seasons and those of the adult pig summer and winter trials, a gradual increase in the pH of the soils underneath the decomposing carcasses was observed (Figures 6.1, 6.8, & 6.14). This increase was notably obvious during the mid- to late stages of decomposition (i.e., advanced decomposition and skeletonization). These recorded observations are in line with the findings of other similar studies (Vass *et al.*, 1992; Benninger *et al.*, 2008; Permenter and MacMahon, 2009; Stokes *et al.*, 2009; Metcalf *et al.*, 2013; Meyer *et al.*, 2013; Olakanye *et al.*, 2017; Szelecz *et al.*, 2018a; DeBryun *et al.*, 2021). As with the pH, the electrical conductivity of the soils underneath the neonate and adult pig carcasses across all seasonal trials was observed to increase as decomposition progressed in comparison to the control soils. These observations are in agreement with the findings of other authors (Aitkenhead-Peterson *et al.*, 2012; Stokes *et al.*, 2009, 2013; Macdonald *et al.*, 2014; Yong *et al.*, 2019; Heo *et al.*, 2021; Taylor *et al.*, 2020).

Although the nutrient (e.g., nitrates, phosphates, ammonia) concentrations in the carcass soils were not investigated, the increase in pH in the soils underneath the carcasses can be attributed firstly to the accumulation of ammonium ions within the soils (Benninger *et al.*, 2008; Meyer *et al.*, 2013; Szelecz *et al.*, 2018a; DeBryun *et al.*, 2021). Several authors have linked the accumulation of ammonium ions in the soils underneath decomposing human or animal remains to the release and build-up of cadaveric fluids (Benninger *et al.*, 2008; DeBryun *et al.*, 2021). Some authors have further suggested that the continuous release and accumulation of cadaveric fluids brings about a shift to anoxia in the soils underneath decomposing carcasses, and this may plausibly explain why accumulated ammonium ions in the soils were not further nitrified (Benninger *et al.*, 2008; Szelecz *et al.*, 2018a). Secondly, the increase in pH of the soils underneath the pig carcasses may be attributed to the movement and feeding activities of

fly larval masses, and Silphid beetles (both adults and larvae) on, within, and underneath the carcasses (Szelecz *et al.*, 2018a; Ilardi *et al.*, 2021).

Fly larval masses are known to secrete proteases which results in the release of the products of proteolysis (e.g., ammonium) within and around decomposing vertebrate remains and other organic substrates (Pinilla *et al.*, 2013; Meneguz *et al.*, 2018; DeBryun *et al.*, 2021). Silphid beetles have been reported to considerably contribute to the increase in pH of soils associated with decomposing carcasses during their feeding and reproduction processes (Ilardi *et al.*, 2021) and this is likely through the secretion of antimicrobial and antifungal compounds (Ilardi *et al.*, 2021). In this study, the significant increase in carcass soil electrical conductivity, in comparison to the control soil, may be attributed to the release of soluble salts, nutrients (i.e., dissolved organic nitrogen), and decomposition fluids from the pig carcasses (Aitkenhead-Peterson *et al.*, 2012; Yong *et al.*, 2019; Taylor *et al.*, 2020). Aitkenhead-Peterson *et al.* (2012) revealed that the dissolved organic nitrogen in the soil was the only chemical constituent that was responsible for the variation in the electrical conductivity of soils underneath decomposing human cadavers. In addition, the significantly high electrical conductivity values of the carcass soils can be linked to the increase in the concentration of charged ions from the available nitrates and phosphates in the soil (Yong *et al.*, 2019).

This study is possibly the first in Africa to examine the changes in the pH of a vertebrate carcass skin at different decomposition stages. In all the neonate and adult pig seasonal decomposition trials, carcass skin pH was observed to increase steadily as decomposition progressed (Figures 6.3, 6.10 & 6.16). This result contradicts the findings of Dibner *et al.* (2019) who reported a decrease in carcass skin pH as decomposition progressed. In this study, during the fresh and sometimes early stages of early decomposition, the carcasses' skin was an acidic environment (Table 6.5; Figures 6.3, 6.10 & 6.16). However, during the late stages of early decomposition and onset of advanced decomposition/skeletonization, an acid to alkaline shift in carcass skin pH was observed (Table 6.5; Figures 6.3, 6.10 & 6.16). This shift in carcass skin pH may be attributed to the anoxic condition of the carcass skin possibly facilitated by the presence of oxygen depleting microbes, and also, the accumulation of ammonium ions due to the absence of oxygen, aggregation, movement and feeding activities of the fly larval masses and maybe, Silphid beetles (Rivers *et al.*, 2011; Meneguz *et al.*, 2018; Dibner *et al.*, 2019; DeBryun *et al.*, 2021; Ilardi *et al.*, 2021).

In accordance with the findings of Chun *et al.* (2015) and Dibner *et al.* (2019), most fly larval masses on the neonate and adult pig carcasses were compact enough to receive a probe between Days 3 to 10 post carcass deployment (Table 6.6). In some trials in this study, fly larval masses were initially in an environment characterized by an acidic pH (Figure 6.4). However, as decomposition progressed, an acid to alkaline shift in larval masses pH was observed (Figure 6.4). This observation agrees with the findings of Junkins *et al.* (2019) who also reported an acid to alkaline shift in larval mass pH as decomposition progressed. In this study, the shift in larval mass pH may be linked to the production and accumulation of ammonium ions facilitated by the anoxic condition of the larval masses (Dibner *et al.*, 2019; Junkins *et al.*, 2019; DeBryun *et al.*, 2021). Some of the larval masses in this study were established from eggs and larvae deposited by blow flies and flesh flies in specific regions of the carcasses with limited oxygen flow such as the oral cavities, ear holes, in/around the abdominal region and between the fore/hindlimbs.

In some trials (e.g., neonate pig spring and summer trials and adult pig summer and winter trials), the larval masses were observed to be largely in an alkaline environment from the onset of their establishment until their dispersal (Figures 6.4, 6.11, & 6.17). These varying observations in larval mass pH may be attributed not only to the nutritional constituents of the carcass tissues on which the larvae were established, but also, the secretion of salivary and gut fluids and digestive enzymes associated with feeding and development of the fly species constituting the larval masses (Kaneshrajah and Turner, 2004; Clark *et al.*, 2006; Day and Wallman, 2006; Rivers *et al.*, 2011; Thyssen *et al.*, 2014; Meneguz *et al.*, 2018). Further laboratory and field studies in different seasons and environmental conditions (e.g., temperature, humidity, and rainfall) would be necessary to improve our understanding of the acid to alkaline shift in pH of larval masses of similar or different fly species and on the same or different tissues of a vertebrate cadaver.

Based on the results obtained from the various seasonal trials conducted in this study, a crime scene investigator could measure the pH and electrical conductivity of the soil underneath decomposing vertebrate remains relative to the control soils and use the estimate to provide some insights in relation to the minimum time since carcass deposition within the Table Mountain region or habitats with similar soil types. In the warm seasonal periods (i.e., summer and autumn seasons), a cadaver soil pH above the neutral pH mark (i.e., >7) and electrical conductivity value of at least 200 μ S/cm greater than the control soil electrical conductivity value, could be used to conservatively indicate a minimum time since cadaver deposition of

around 5 to 6 days. In the spring and winter seasons, considerable variations should be expected not only in the timing of the acid to alkaline shift in cadaver soil pH, but also, the attainment of a carcass soil electrical conductivity value at least 200 μ S/cm greater than the control soil electrical conductivity value. Consequently, the soil pH and electrical conductivity data based on the benchmark suggested above should be used with some degree of caution when attempting to estimate the minimum time since cadaver deposition in any season.

With respect to the carcass skin pH, differences exist monthly and seasonally, and these differences may be attributed to the fluctuations in ambient temperature, relative humidity, and rainfall patterns and larval masses sizes. In some seasonal trials (e.g., summer and autumn seasons), an acid to alkaline shift in larval mass pH could potentially provide some clues in relation to the time since cadaver deposition as suggested by Chun *et al.* (2015). However, in some trials across the summer and winter seasons (e.g., neonate pig summer trials, adult pig summer and winter trials) larval masses were majorly an alkaline environment from the onset of their establishment until their dispersal. It is noteworthy that measurement of larval mass pH can only be taken when the larval mass is large enough to receive a probe, and because of this, there might be some limitations to the utilization of larval masses pH in estimating the minimum deposition time of a cadaver especially during the cold seasonal periods that are often characterized by limited fly activity and minimal larval mass formations.

The results from this study suggest that carcass size has no significant impact on the changes in the pH and electrical conductivity of carcass-associated soils in the summer seasonal period (Figure 6.12). This may be attributed to the lack of significant differences in the ambient temperature between the adult and neonate pig summer decomposition trials. Conversely, in the winter seasonal period, carcass size played a significant role, as the pH and the electrical conductivity of the adult pig carcass were significantly higher than that of the neonate pig carcasses (Figure 6.18). This observation was not surprising considering the extended decomposition duration of the adult pig carcass in the winter season. Although not investigated in this study, the significantly higher pH and electrical conductivity observed in the soil underneath the adult pig carcass may be attributed to the enormous amount and concentration of total nitrogen and ninhydrin-reactive nitrogen deposited into the underlying soil from the carcass (Spicka *et al.*, 2011; Fancher *et al.*, 2017). Furthermore, the high pH and electrical conductivity of the adult pig carcass soil may be attributed to the large surface area of the adult pig coupled with the large volume and number of fly larvae feeding on, around and underneath

the carcass all of which contributes to the discharge of more decomposing fluids from the carcass into the surrounding environment (Spicka *et al.*, 2011; Weiss *et al.*, 2015).

During the adult pig summer and winter trials, a considerably large “sea-wave” of fly larvae was seen on multiple occasions, actively feeding, and moving all over the carcass, and in the region between the carcass and the underlying soil surface. This, in turn, brought about the rapid opening of the carcass, and subsequently, the production, accumulation, and deposition of maggot- and carcass associated fluids. In each of the neonate pig winter trials, the size and volume of the larval masses were between 2 – 3 cm² in area, and they were mostly confined to selected regions of the carcasses and carcass-associated soils. Equally important, carcass size was found to significantly influence the pH of the carcass skin in the summer and winter seasonal periods (Figures 6.13 & 6.19). This observation, again, is related to the large surface area of the adult pig carcasses, which supports the feeding activities of more larval masses. Furthermore, the widespread movement of the larval masses alongside the deposition of maggot- and carcass associated fluids all over the carcass may be a contributing factor to the variation in carcass skin pH between the adult and neonate pig carcasses.

The results yielded by the generalized additive models provide strong evidence that ADD, which is the cumulative sum of average daily temperature, is the most important environmental variable influencing the changes in carcass skin pH and the pH and electrical conductivity of carcass-associated soils (Figures 6.5 – 6.7) (Vass *et al.*, 1992). In line with the findings of Giles *et al.* (2020), ambient relative humidity also exerts a considerable influence on the changes in carcass skin pH and carrion-impacted soil chemistry as decomposition progresses especially in the warm seasonal period (Figures 6.5 – 6.7). Principally, the adjusted R² values from the generalized additive models seem to suggest that the changes in carcass skin pH and carcass soil pH and electrical conductivity is a complex process linked with several other environmental variables needing further investigation. Some of these variables may include soil type, series, texture, moisture, soil soluble salt and nutrient concentrations and soil microbial composition (Metcalf *et al.*, 2013; Aitkenhead-Peterson *et al.*, 2015; DeBryun *et al.*, 2021; Taylor *et al.*, 2023). These variables may have also contributed to the significant variations in the pH and electrical conductivity of the control soils. Above all, the results from this study provide soil chemistry data that can assist forensic investigators in the estimation of the PMImin and time since cadaver deposition within the Table Mountain region or regions with similar soil types.

Chapter 7: Synthesis, limitations, and conclusion

7.1. Synthesis

This study explored how the diversity, abundance and successional patterns of carrion-associated insects differ in each month of the year, and cumulatively, across the four seasons of one year within Table Mountain National Park in Cape Town, South Africa, using neonate pig carcasses. Furthermore, this study explored whether significant differences exist in the insect species breeding on the pig carcasses deployed in each month across a single season. The entomological data generated from the neonate pig trials were further compared to similar data generated from the adult pig trials and those collected from decomposing human remains, as documented in forensic entomological case reports. Furthermore, this study investigated how the pH of the carcass skin and larval masses may change as decomposition progressed across the four seasons of one year. Also, it explored how the pH and electrical conductivity in the underlying and surrounding soil would respond to decomposition fluids and materials originating from decomposing neonate and adult pig carcasses across several seasonal cycles.

7.2. Study limitations and future research

Despite the valuable data generated in this study, there are several limitations which should be considered when interpreting the results. These limitations are discussed below:

1. Caging of carcasses and non-exposure to local vertebrate scavengers

By caging the carcasses, locally occurring vertebrate scavengers are automatically prevented from interacting with the pig carcasses and other carcass-associated insects. Vertebrate scavengers such as the Cape mongoose have been documented to be attracted to decomposing vertebrate remains in different seasons of the year within the Western Cape Province (Finaughty, 2019; Spies, 2022). These vertebrate scavengers have been shown to cause significant disarticulation and scattering of several parts/appendages of the carrion which in turn can complicate their recovery process and process of carrion decomposition. Additionally, some vertebrate scavengers can feed on a vertebrate carrion, and subsequently create oviposition sites attractive and suitable for invertebrate scavengers (i.e., insects) to lay their eggs and have direct access to the soft tissues of the body (Flint *et al.*, 2022).

While most studies focused on establishing baseline data on the insect assemblages and their successional patterns on decomposing vertebrate remains have excluded vertebrate scavengers by placing their experimental subjects in purpose-designed experimental cages, this

does not reflect what is expected in actual forensic cases. While cages are necessary to limit loss of research specimens, further research is necessary to design studies which can take into account the impact of vertebrate scavenger activities on the diversity and abundance of carrion-associated insect species and changes in soil chemistry.

2. Lack of clothing

In this study, only unclothed pig carcasses were deployed. Within the Western Cape Province, several types of body coverings (i.e., clothing) have been reportedly found on decomposing human cadavers during forensic anthropological and entomological case work analysis (Spies *et al.*, 2020; Baliso *et al.*, 2019, 2023). The influence of clothing on the rate/pattern of decomposition and insect colonization is conflicting based on published results in the literature (Kelly, 2006; Card *et al.*, 2015; Matuszewski *et al.*, 2016; Mashaly *et al.*, 2019b; Spies *et al.*, 2020). Some studies have shown that the presence of clothing speeds up the rate of carrion decomposition by accelerating the development of insects on the carrion (Cahoon, 1992). Other studies have shown that clothing decelerates the rate of decomposition by retaining moisture and preventing adult flies from laying their eggs or larvae directly on the soft tissues of the carrion (Goff, 1992; Kelly *et al.*, 2011; Spies *et al.*, 2020).

With respect to carrion entomofauna, results from the findings of Matuszewski *et al.* (2016) revealed that clothing has little impact on the insect species attracted to decomposing pig carcasses. Kelly *et al.* (2011) revealed that the presence of clothing altered the distribution of maggot masses feeding on pig carcasses, but only during the warm seasonal periods. Interestingly, Mashaly *et al.* (2019b) revealed that clothing had no impact on both the decomposition rate and presence of insects on decomposing rabbit carcasses. Owing to the unclear nature of the impact of clothing on the decomposition rate/pattern of vertebrate remains and carrion-associated insects, and the lack of empirical studies on the influence of clothing on carrion-associated insect species and changes in soil chemistry within this region, it is important to apply caution when extrapolating the results from this study (due to the utilization of unclothed pig carcasses) to actual forensic cases within the region.

3. Use of stillborn and frozen-thawed pig carcasses

To gain a large enough sample for monthly tests, it was necessary to utilize frozen neonate pig carcasses. This is not uncommon in the forensic entomology literature (Payne, 1965; Archer, 2004; Cruise *et al.*, 2018; Munro *et al.*, 2019; Blonar and Prada-Tiedemann, 2020; Ziogos *et*

al., 2023). However, the utilization of such specimens may alter the true PMI of the animals as it is unknown exactly when such animals died. Furthermore, the effects of freezing and subsequent thawing have not been fully investigated. Nevertheless, the results generated in the neonate and adult pig decomposition trials had similar insect diversity. The discrepancies in insect abundance and changes in carcass skin and soil chemistry are attributed to carcass size, although the effect of freezing cannot be entirely ruled out.

4. Placement of pig carcasses in partially open-bottomed plastic baskets

In this study, specifically in the neonate pig trials, the pig carcasses were placed in partially open-bottomed plastic baskets, and thus were not fully in contact with the underlying soil. While the open-bottomed nature of the baskets still allowed insect access (e.g., beetles and ants), and the seepage of decomposition fluids and organic materials into the underlying soils from the carcasses, it is not envisaged that this would have a major effect on the results of chemical analyses of the soil. This is supported by the results of the direct comparisons of the soil chemistry data generated between the neonate and adult pig carcasses in the summer season. Nevertheless, it is worth mentioning that the baskets could have resulted in some delay in insect access, and seepage of decomposition fluids from the neonate pig carcasses into the underlying soils, which in turn, could have influenced the results obtained.

5. Impact of purpose-designed fly netting on carrion decomposition, arrival and oviposition/larviposition of flies and other insects

To obtain a high resolution of insect species that are attracted to decomposing vertebrate remains within the Western Cape Province and Table Mountain region, this study utilized purpose-designed tent-like brown nettings to trap and collect the adult flying insect species that were attracted to the decomposing neonate and adult pig carcasses. Admittedly, the purpose-designed fly nettings generated valuable results specifically in relation to the insect species that fed on the carcasses, and those that deposited their eggs and larvae, preyed on, and parasitized the immature stages of other carrion-associated insects. However, it is important to state that the deployment of the fly nettings above the carcasses may have influenced the process of carrion decomposition by providing some degree of shade over the carcasses.

Based on the results obtained following the complete rearing of all the eggs/larvae collected from the carcasses, only a handful of the fly species trap/collected, deposited their eggs/larvae on the carcasses in the neonate and adult pig trials. Nonetheless, the unintentional “caging” and associated buzzing sounds of several flies (including those that are of the same genus/species)

within the fly nettings, may have attracted other flies that are conspecifics/congeners and/or triggered the process of oviposition/larviposition on the carcasses.

6. Frequency of collection

Efforts were made to collect entomological, decompositional, and soil data every day. However, data collection was not possible on some days due to unfavorable weather conditions (e.g., excessive rainfall), transport-related constraints, and fatigue/illness on the part of the author. Also, due to loss of data and associated technical issues, some gaps were noted in the ambient temperature and relative humidity data on some days. This was rectified through generalized additive modelling to fill in the gaps. The continuous sampling of soils underneath the pig carcasses may have triggered early aeration within the soil columns (Fancher *et al.*, 2017). This may potentially affect the extrapolation of the results obtained from this study in the estimation of the PMI_{min} or time since cadaver deposition when using soil samples collected underneath previously undisturbed human remains found in open terrestrial environments (Fancher *et al.*, 2017). For this reason, it is suggested that some degree of caution should be used when interpreting these results.

7. Sample size and duration of the study

In this study, two neonate pig carcasses were utilized to generate baseline decomposition, insect successional and soil chemistry data on a monthly temporal scale across the four seasons of a single year. Also, this study utilized singly deployed adult pig carcasses across the summer and winter seasons of a single year to generate forensically relevant data (as with the neonate pig trials) that can be compared to the findings of the neonate pig trials, and further applied to local forensic cases within the region. While the results generated in this study are noteworthy, it is important to note here the need for further baseline studies with multiple singly deployed adult pig carcasses, across several seasons, years, and locations within the Western Cape Province. This will facilitate the validation of the results generated in this study, and verification of the role of seasonal/annual variations on the colonization pattern, and oviposition/larviposition of carrion-associated entomofauna on decomposing vertebrate remains.

8. Lack of decomposition and insect data in April 2021 due to a wildfire

The wildfire that occurred on the 18th of April 2021, specifically within and around the vicinity of Table Mountain National Park (BBC News, 2021) and University of Cape Town (University of Cape Town News, 2021) significantly impacted on the data collection during the neonate

pig trials. The wildfire resulted in the complete lockdown of the National Park by the South Africa National Park and Table Mountain National Park Authorities, and this subsequently led to the temporary cessation of the field trials and loss of data collection for the month of April.

7.3. Primary findings

While all the results presented in this study are important, the primary findings are summarized below.

1. Insect species colonizing and breeding on decomposing carcasses in each month of a single season were different

The data presented in this thesis represent one of the first contributions to the knowledge of insect diversity, abundance, and successional patterns on decomposing animal carcasses in each month of a single season. This study demonstrated that considerable differences exist with regard to the first insect colonizers of decomposing carcasses in each month of a single season. As documented in Chapter 3, in the spring season neonate pig trials, for instance, the immature stages of *Ch. chloropyga* and Sarcophagidae gen. sp. were recorded on the carcasses deployed in all the three trials. However, the immature stages of both *Ca. vicina* and *Ca. croceipalpis* were only recorded on the neonate pig carcasses deployed in the early spring trial. Similar observations were also documented in the autumn season neonate pig trials where the eggs and larvae of *H. fernandica* were noted on the carcasses deployed in the mid- and late autumn seasonal periods, but not in the early autumn.

There were also some noteworthy differences in the insect species that bred on the neonate pig carcasses in each month across the summer and winter seasons. Based on these results, it is strongly recommended that researchers should investigate whether differences occur in the first insect colonizers of decomposing carcasses not only between seasons, but also, within each month of a single season. This will reduce the possibility of making inaccurate assumptions especially when creating baseline seasonal data on the diversity, abundance and successional patterns of carrion-associated insect species and their roles in PMImin estimations in each geographical region.

2. Successful colonization and oviposition of *Ca. vicina*, *Ca. croceipalpis*, *Ch. megacephala* and *H. fernandica* on decomposing pig and/or human remains

The results generated from this study as documented in Chapters 3 and 5 revealed for the first time in South Africa after over 3 decades, the successful colonization, and oviposition of *Ch.*

megacephala and *H. fernandica* on decomposing animal and human remains. Although Parry *et al.* (2016) and Williams and Villet (2019) recorded the presence of adult *Ch. megacephala* in the Gauteng and Eastern Cape Provinces respectively, their studies only focused on generating baseline data on the assemblage and distribution of forensically important flies attracted to baited fly traps. In agreement with other authors, there is a need for the validation of the fly assemblage data generated using baited fly traps with whole carcasses (Villet *et al.*, 2017). This is further supported by the findings of other studies where they suggested that the use of insect diversity and abundance data from baited fly traps in forensic investigations should be done with caution as they do not accurately represent the fly species occurrence and general insect assemblage attracted to vertebrate carrion (Hepburn, 1943; LeBlanc *et al.*, 2021).

Both *Ca. vicina* and *Ch. megacephala* are invasive blow fly species in South Africa, and their ability to breed on both human and animal remains in the spring and winter seasons (for *Ca. vicina*) and summer season (for *Ch. megacephala*) within the Western Cape Province as observed in this study is noteworthy. Furthermore, it suggests that they can be used in PMImin estimations within the Province. At the time of writing, no other decomposition or insect successional studies in other Provinces, apart from the findings of Prins (1982) in the Western Cape Province, have documented the presence of the immature stages of *Ch. megacephala* on decomposing vertebrate remains. For this reason, it is not out of place to suggest that *Ch. megacephala* can be used as a potential indicator of body translocation from one Province to another.

The successful oviposition of *H. fernandica*, *Ch. megacephala* and *Ca. croceipalpis* on the deployed neonate pig carcasses within the Table Mountain region suggests that these blow fly species could potentially be used as indicator species of body translocation especially between Provinces and within the Western Cape Province/City of Cape Town. This is their immature and/or adult stages have not been recorded on human/animal remains within the past 3 decades. Finaughty (2019) did not observe or report the adults and immature stages of these three blow fly species on decomposing pig carcasses in two contrasting environments (i.e., open and thicketed/shaded) in the Cape Flats Dune Strandveld area of the City of Cape Town. The fact that some of the insect species recorded in this study were not reported in Finaughty (2019) validates the findings of other studies where differences in insect species assemblages on decomposing vertebrate remains in different geographical areas or localities within the same region were noted (Cruise *et al.*, 2018; Díaz-Aranda *et al.*, 2018).

3. Necrophagous, predatory and parasitic behavior of ants (*Crematogaster* cf. *liengmei*) and wasps (*Alysia manducator*)

In Chapters 3 and 4, the necrophagous, predatory, and parasitic behaviors of *Crematogaster* cf. *liengmei* and *A. manducator* on carrion-associated entomofauna and the carrion itself were documented. The flesh-eating behavior of *Crematogaster* cf. *liengmei* along with the predatory and parasitic behaviors of both *Crematogaster* cf. *liengmei* and *A. manducator* on the insects breeding and feeding on the decomposing vertebrate remains may alter the process of carrion decomposition, and potentially compromise or interfere with the widely accepted method of utilizing the immature stages of insect species recovered from vertebrate remains as entomological evidence in PMImin estimations. The bitemarks and skin lesions inflicted by the feeding activities of *Crematogaster* cf. *liengmei* on the neonate and adult pig carcasses as documented in Adetimehin *et al.* (2022) and Chapter 5 respectively can be misinterpreted as ante- or peri-mortem traumatic injuries by inexperienced forensic investigators. Interestingly, previous decomposition studies (Finaughty, 2019; Spies 2022) across multiple seasons and localities within the Western Cape Province did not report any of the above-mentioned observations. Because of the forensic implications of the presence of these two insect groups on and around decomposing vertebrate remains, it is crucial for forensic practitioners involved in the collection and analysis of vertebrate remains to take note of these two insect groups during medico-legal forensic and anthropological investigations. Also, further detailed studies are needed to evaluate the impact of ants and wasps on the rate or pattern of carrion decomposition and development of carrion-associated insects within this region.

4. Successful colonization and larviposition of Sarcophagidae species on the neonate pig and human remains

In this study, the larvae of Sarcophagid flies were recorded on the neonate pig carcasses and human remains in all seasons within this region. These observations clearly show that Sarcophagid flies are important entomological candidates that can provide valuable information relating to the PMImin of decomposing vertebrate remains. However, owing to the lack of sufficient developmental data on Sarcophagid flies in various parts of the world, a fact which is further complicated by the difficulties associated with their identification to the species level, the utilization of this insect group in minimum time since death estimations have not been significantly explored. As with the blow fly species mentioned above, the immature stages of Sarcophagid flies were not observed on the pig carcasses deployed by Finaughty

(2019) across several seasons. For this reason, further studies are needed on the identity, distribution pattern and developmental rate of Sarcophagidae species in this region and beyond.

5. Carcass size influences skin and soil chemistry, and insect species assemblage

The results from this study indicate that carcass size exerts a significant impact on the number of insect species and individuals attracted to decomposing vertebrate remains. Furthermore, the outcomes of this study revealed that carcass size influences certain changes in the chemical properties of the carcass skin and underlying soil. This study revealed that carcass size exerts an influence on the presence or absence of specific late arriving insect species. For instance, in the adult pig summer and winter seasonal decomposition trials, members of the family Cleridae were observed to colonize the carcasses in considerable numbers during the late stages of decomposition. This was also the case in other similar studies in South Africa utilizing large sized pig carcasses (Kelly *et al.*, 2009; Finaughty, 2019). In contrast, during the neonate pig summer trials, the number of Cleridae individuals observed were substantially small, while none were observed on the carcasses in the spring, autumn, and winter season trials. In Chapters 3, the complete absence and underrepresentation of Clerid beetles on small-sized carcasses agrees with what has been reported in the literature (see Matuszewski *et al.*, 2016). Based on the findings of this study, it is important to empirically examine how carcass body size/mass affects the assemblage of carrion-associated insects and soil chemistry across multiple seasons.

6. Similarity in the insect assemblages colonizing the pig carcasses and human remains

In this study, there were substantial similarities in the adult and immature insect species collected from the pig carcasses used in the experimental field trials and those collected from the human remains, across several decomposition stages and seasons. These similarities suggest that both neonate and adult pig carcasses can be used to generate valuable baseline data on carrion-associated insect species across several months or seasons within a specific region or locality. Despite the valuable data generated from the experimental field studies, the contribution of forensic entomology in forensic investigations within the Province might still be undermined if there are no improved interactions and collaborations between the forensic pathologists and entomologists within the region. Given the limited availability of consultant forensic entomologists, it will be of benefit to employ forensic entomologists in the Forensic Pathological Services in order to expand the utilization of entomological evidence in medico-legal investigations and time since death estimations.

7. ADD, relative humidity and rainfall do not sufficiently explain the changes in carcass skin and soil chemical responses to decomposing vertebrate remains

The results from the GAM analyses highlight the influence of ADD and ambient relative humidity on the changes in the carcass skin pH and carrion-impacted soil pH and electrical conductivity. Yet, it revealed that rainfall played no significant role in the variations in pH and electrical conductivity of carrion-associated soils. These results further suggest that there are other factors that might be influencing the process of decomposition and changes in carcass skin and soil chemistry. It is possible that the texture, moisture, nutrient, and microbial composition of the soil may have contributed to these variations. Nevertheless, more studies are needed to fully understand the biochemical responses of different soil types to the decomposition fluids and materials associated with the remains of different models (e.g., rats, rabbits, humans etc.). It would also be useful to evaluate the impact cadaver decomposition on the microbiome of the carcass skin, larval masses, and underlying soil across several seasons. This may reveal interesting trends that can contribute meaningfully to time since death estimations within this region and beyond.

7.4. Secondary/Incidental findings

As part of the results observed during the field trials there were some additional results that were unexpected. These findings are summarized below.

1. Incidental observation of bone scavenging by *Crematogaster cf. liengmei*

Although not the major focus of this study, while the field data collection was ongoing, specifically in the adult pig winter seasonal trial, several individuals of *Crematogaster cf. liengmei* were observed on and around a bone incidentally found lying along the road on the Rhodes Memorial Street, within the Table Mountain National Park. The location of the bone was about 50 meters from the autumn season neonate pig carcasses deployment sites, where individuals of the same ant species were observed inflicting bite marks and numerous skin lesions on the flesh of the carcasses. Upon inspection of the bone, several *Crematogaster cf. liengmei* individuals were seen actively feeding on and removing bone particles and soft tissue remnants on the bone. A closer look at the epiphyseal ends of the bone revealed several striae/furrows presumed to be inflicted by the feeding activities of the ants. In addition, a few individual ants were observed removing some bony and fleshy particles away from the bone.

Remarkably, the morphology of the striae/furrows observed on the bone had some similarity to the bite marks inflicted by the same ants on the external part of the right ear of the adult pig carcass (see Chapter 4), a day after colonization by *Crematogaster* cf. *liengmei* individuals within the same environment during the winter season. Based on these preliminary observations, it is suggested that further studies should empirically investigate the post-mortem modification by ants of different bones or skeletal materials of different animal models in varied states of preservation, to document how the presentation of these modifications might differ across seasons and with varied exposure durations, and to compare with the striations/furrows inflicted by other insect groups (e.g., beetles and termites) and vertebrate scavengers. This will reveal useful information that can be used in differentiating post-mortem excoriations and other forensically significant traumatic striations on human and animal skeletal remains during both forensic and anthropological investigations. Detailed explanation and photographic evidence of this observation has been documented in Adetimehin *et al.* (2023b).

2. Unidirectional migration of blow fly larvae in the direction of the flow of decomposition fluids

The observed unidirectional and south-westerly migration of several blow fly larvae together with the decomposition fluids from the carcass documented in Chapter 6 was an unexpected observation in this study that needs further attention. Although a few authors (Lewis and Benbow, 2011; Heinrich, 2013; Lashley *et al.*, 2018; Goddard *et al.*, 2020) have documented similar observations and have attempted to explain the reasons behind this phenomenon, this behavior by blow fly larvae is worthy of consideration during forensic investigation as it can increase the lateral spread of decomposition fluids from the carrion into the surrounding environment. This can further confuse crime scene investigators and even human cadaver detection dogs, especially when attempting to locate specific cadaver deposition sites. Furthermore, when encountered by inexperienced crime scene investigators, it could possibly lead to an erroneous conclusion that the cadaver or one of its appendages was dragged from one location to another by large sized vertebrate scavengers or assailant(s) as part of the cadaver concealment process. Owing to this, it is crucial to investigate the ecological factors triggering the mass dispersal of blow fly larvae together with decomposition fluids from vertebrate remains in different ecological environments.

7.5. Conclusion

This study has generated the much-needed baseline data on the diversity, abundance and successional patterns of insect species associated with decomposing vertebrate remains in each month across all the seasons within the Table Mountain region. In terms of its contribution to scientific literature, this study has shown that insect species, especially those that deposit their eggs and/or larvae on decomposing vertebrate remains, differ in each month within some seasons. Furthermore, this study has revealed for the first time after 3 decades, the successful colonization and oviposition of three blow fly species viz. *Ch. megacephala*, *Ca. croceipalpis* and *H. fernandica* on decomposing human and/or animal remains and further suggest that they can be used as indicators of body translocation from one Province to another. Further research directly exploring the current geographical distribution of these blow fly species and their abilities to deposit their eggs on decomposing vertebrate remains in South Africa is necessary. Lastly, this study clearly highlights the similarity in the insect species colonizing decomposing pig and human cadavers across several seasons and the need for effective collaboration between forensic entomologists and other forensic practitioners in the utilization of entomological evidence in medico-legal investigations.

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Appendices

Appendix A: Ethical Approval Letters and Permits



03 March 2020

Dr M. Heyns
Department of Pathology
Division of Forensic Medicine & Toxicology
Room 5.12
Falmouth Building FHS

Dear Dr Heyns

PROTOCOL TITLE: Insect fauna and their pattern of succession on decomposing stillborn pig carcasses: Implication for Forensic Entomology in Western Cape, South Africa (MSC Candidate- Mr Adeyemi Adetimehin)

FHS AEC REF NO: 019_036

Thank you for submitting your protocol to the Faculty of Health Sciences (FHS) Animal Ethics Committee (AEC) for review.

I am pleased to inform you that the FHS AEC has **approved** your protocol, which will terminate on **31 March 2023**.

Number of animals & species: 24 stillborn pig carcass, Unpredictable number of insects

Please quote the FHS AEC REF NO (above) in all future correspondence.

Please note that the approval of this protocol imposes the following obligations on the principal investigator (PI):

1. To submit an annual mandatory progress report. The first annual report for this protocol is due on **28 February 2021**. The forms can be accessed from <http://www.health.uct.ac.za/fhs/research/animalethics/forms>
2. To submit a final mandatory report on the **31 March 2023**, please access the final report form from: <http://www.health.uct.ac.za/fhs/research/animalethics/forms>
3. Ensuring that all study participants perform within the confines of the procedures and experimental design of the protocol as approved, or as amended.

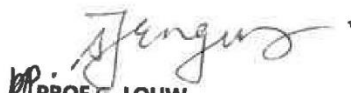
AEC REF# 019_036

Figure A1: A copy of page one of the approval letter for application with protocol number 019_036 from the University of Cape Town's Faculty of Health Sciences Animal Ethics Committee.

4. Ensuring that all study participants comply with all applicable national legislation, UCT policies, FHS AEC policies and standard operating procedures (SOPs) and national standards (SANS 10386: 2008).
5. Ensuring that you as the PI immediately alert the FHS AEC to any event involving the welfare of the animals which has occurred during the course of the study, as well as the actions that were taken to respond to these events.
6. Ensuring that you as the PI alert the FHS AEC to any new or unexpected ethical issues that arose during the course of the study, and how these issues were addressed.
7. Ensuring that all study participants are registered with or have been authorised by the South African Veterinary Council (SAVC) to perform the procedures on animals or will be performing the procedures under the direct and continuous supervision of SAVC-registered veterinary professionals or SAVC-registered para-veterinary professionals.
8. If the PI or any study participant is in any way uncertain how to respond to any of these obligations or deal with any of the issues referred to above, they must consult with FHS AEC.
9. All animals found dead must be reported to the RAF on the appropriate form:
<http://www.health.uct.ac.za/fhs/research/animalethics/forms>
10. All animals found in distress must be reported to the RAF on the appropriate form.

My best wishes for a successful research and /or teaching endeavour.

Yours sincerely


PP. PROF G. LOUW
CHAIR, FHS AEC

AEC REF# 019_036

Figure A2: A copy of page two of the approval letter for application with protocol number 019_036 from the University of Cape Town's Faculty of Health Sciences Animal Ethics Committee.



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Animal Ethics Committee



Room G50 Old Main Building
Groote Schuur Hospital
Observatory 7925

Website: www.health.uct.ac.za/fhs/research/animalethics/forms

10 December 2021

Mr Calvin Mole

Division of Forensic Medicine and Toxicology
Department of Pathology
Faculty of Health Sciences
University of Cape Town

Dear Mr Mole

PROTOCOL TITLE: *Insect succession and changes in the soil pH and electrical conductivity associated with decomposing pig carcasses on the Table Mountain National Park of the Western Cape Province of South Africa (PhD Student- Mr Adeyemi Daniel Adetimehin)*

FHS AEC REF NO: 021_021 PREV 019_036

Thank you for submitting your amended protocol to the Faculty of Health Sciences (FHS) Animal Ethics Committee (AEC) for review.

I am pleased to inform you that the FHS AEC has **authorised** your protocol, which will terminate on **30 December 2024**.

Number of animals & species:

- 2 Adult pigs
- Wild insects (numerous)

Please quote the FHS AEC REF NO (above) in all future correspondence.

Please note that the authorisation of this protocol imposes the following obligations on the principal investigator (PI):

1. To submit an annual mandatory progress report. The first annual report for this protocol is due on **28 February 2022**. The forms can be accessed from <http://www.health.uct.ac.za/fhs/research/animalethics/forms>
2. To submit a final mandatory report on the **30 December 2024**, please access the final report form from: <http://www.health.uct.ac.za/fhs/research/animalethics/forms>

AEC REF# 021_021 PREV 019_036

Figure A3: A copy of page one of the approval letter for application with protocol number 021_021 from the University of Cape Town's Faculty of Health Sciences Animal Ethics Committee.

3. Ensuring that all study participants perform within the confines of the procedures and experimental design of the protocol as authorised, or as amended.
4. Ensuring that all study participants comply with all applicable national legislation, UCT policies, FHS AEC policies and standard operating procedures (SOPs) and national standards (SANS 10386: 2008).
5. Ensuring compliance with DAFF Section 20 requirements.
6. Ensuring that you as the PI immediately alert the FHS AEC to any event involving the welfare of the animals which has occurred during the course of the study, as well as the actions that were taken to respond to these events.
7. Ensuring that you as the PI alert the FHS AEC to any new or unexpected ethical issues that arose during the course of the study, and how these issues were addressed.
8. Ensuring that all study participants are registered with or have been authorised by the South African Veterinary Council (SAVC) to perform the procedures on animals or will be performing the procedures under the direct and continuous supervision of SAVC-registered veterinary professionals or SAVC-registered para-veterinary professionals.
9. If the PI or any study participant is in any way uncertain how to respond to any of these obligations or deal with any of the issues referred to above, they must consult with FHS AEC.
10. All animals found dead must be reported to the RAF on the appropriate form:
<http://www.health.uct.ac.za/fhs/research/animalethics/forms>
11. All animals found in distress must be reported to the RAF on the appropriate form.

My best wishes for successful research and /or teaching endeavour.

Yours sincerely



PROF. G. LOUW
CHAIR, FHS AEC

AEC REF# 021_021 PREV 019_036

Figure A4: A copy of page two of the approval letter for application with protocol number 021_021 from the University of Cape Town's Faculty of Health Sciences Animal Ethics Committee.



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee



Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

23 August 2021

HREC REF: 528/2021

Dr M Heyns

Division of Forensic Medicine
Falmouth Building-FHS
Email: Marise.Heyns@uct.ac.za
Student: ADTADE003@uct.ac.za

Dear Dr Heyns

PROJECT TITLE: INSECT SUCCESSION AND CHANGES IN THE SOIL PH AND ELECTRICAL CONDUCTIVITY ASSOCIATED WITH DECOMPOSING PIG CARCASSES ON THE TABLE MOUNTAIN NATIONAL PARK OF THE WESTERN CAPE PROVINCE OF SOUTH AFRICA-PHD CANDIDATE- ADEYEMI DANIEL ADETIMEHIN-SUB-STUDY LINKED TO 036/2014

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020; 06 July 2020 & 01 July 2021.

Approval is granted for one year until the 30 August 2022.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: Adeyemi Adetimehin will also be involved in this study.

Please quote the HREC REF 528/2021 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

HREC/REF 528/2021sa

Figure A5: A copy of page one of the approval letter for application with protocol number 528_2021 from the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee.

Yours sincerely



PROFESSOR M. BLOCKMAN

CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938

NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC/REF 528/2021sa

Figure A6: A copy of page two of the approval letter for application with protocol number 528_2021 from the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee.

To develop, expand, manage and promote a system of sustainable national parks that represent biodiversity and heritage assets, through innovation and best practice for the just and equitable benefit of current and future generations.			
Permit number: CRC/2020-2021/024--2019/V1			
CAPE RESEARCH CENTRE P.O. Box 216, STEENBERG, 7947 Tel: +27 (0)21 713 7511; Fax: +27 (0)21 712 0131			
Research Permit: TABLE MOUNTAIN NATIONAL PARK		29 June 2020	
Dr. Marise Heyns - "Insect fauna and their pattern of succession on decomposing pig carcasses: Implications for Forensic Entomology in Western Cape, South Africa" <u>Co-workers:</u> Adeyemi Daniel Adetimehin, Wilmari Uys, Kyle Sieghard Kulenkampff,			
Division of forensic medicine and toxicology University of Cape Town, Observatory			
This permit is valid from 29 June 2020 until 31 June 2021. The approval is subject to the following conditions. The Park Management staff must be contacted prior to entry into the park (see list of staff members below). You are allowed access to the Northern section of the park (please see attached map for restricted areas).			
Standard Conditions: • The use of non-demarcated areas will lead to the disturbance of animals and eco-systems, trampling of vegetation and soil erosion and only the use of accepted pathways and areas is therefore permitted, UNLESS BY SPECIAL ARRANGEMENTS. PLEASE CONTACT THE PARK MANAGEMENT STAFF IF RESTRICTED AREAS NEED TO BE ACCESSED. • No damage shall be permitted to any natural vegetation, environment or property. • Animals may not be disturbed in any way. • Uncontrolled vehicle access and parking could cause damage to vegetation and soil erosion and therefore only the use of approved vehicles routes and parking areas is allowed. • Fires can cause loss of vegetation, soil erosion and life and therefore fires, and braai's are not permitted unless in dedicated braai areas. • Remove all rubbish and waste as it has an effect on the health of visitors, animals and plants. • Other visitors to the area and or neighbours may not be hindered in any way. • Pollution affects the health and safety of animals, plants, visitors and neighbours and is not permitted. • Excessive noise affects animals (e.g. birds nesting in the areas), visitors and or neighbours and is not permitted. • Your permit and identification must be retained and kept on your person at all times, and produced on request. • The areas under the control of SANParks are used entirely at your own risk. South African National Parks, its Board, directors, employees and agents are not liable for any loss or damage to the property or possession of any guest or participant (or accompanying minor) whether such damage is caused by the negligent act or omission of South African National Parks; arising from death or any bodily injuries of whatsoever nature sustained by a guest or participant (or accompanying minor) whether such injuries are caused by the negligent act or omission by South African National Parks, and/or by the defective functioning of any apparatus. The guest or participant and/or his/her/their estate hereby indemnifies South African National Parks against any claim, action, judgment, costs and/or expenses which may be made against South African National Parks and as may in any way be related to the above. The onus lies with the company or applicant to ensure that they are adequately insured.		addo elephant agulhas augrabies bontebok camdeboo golden gate highlands karoo kgalagadi transfrontier knysna lake area kruger mapungubwe marakele mokala mountain zebra namaqua table mountain tankwa karoo tsitsikamma richtersveld west coast wilderness	
643 Leyds Street Muckleneuk Pretoria	PO Box 787 Pretoria 0001	tel: 012 426 5000 fax: 012 343 0905	central reservations: 012 428 9111 reservations@parks.co.za www.parks-sa.co.za

Figure A7: A copy of page one of the research study permit (for the years 2020 – 2021) within the vicinity of the Table Mountain National Park from the South African National Parks' Authorities.

To develop, expand, manage and promote a system of sustainable national parks that represent biodiversity and heritage assets, through innovation and best practice for the just and equitable benefit of current and future generations.



- Please note that you (your delegates, staff etc) are subject to the conditions set in terms of Section 86(1) of the National Environmental Management Act (107 of 1998) and the National Environmental Act: Protected Areas Act (Act 57 of 2003) for the duration of your stay in the National Park. Your attention is specifically drawn to sections 64(1) (a), (b) & (c) which refers to penalties in terms of the Act.
- SANParks staff's instructions, notices, regulations and signs must be complied with.
- The activity shall be restricted to the area applied for.
- Gate and operating times to be complied with.
- NO PETS ALLOWED

Special Conditions:

- The researches may work in the area of the park surrounding UCT.
- Every month, two stillborn pig carcasses of similar weights will be placed individually in purpose designed fly traps a minimum of 30 meters apart. Thereafter, pitfall traps will be placed at four different points (north, south, east, west) around each of the fly trap at a minimum of 1 meter.
- Pig carcasses must be appropriately hidden and camouflaged in brown netting and vegetation.
- Adult flying and ground-crawling insects attracted to the stillborn pig carcasses will be collected.

Area	Park Management Staff	Telephone Number:
TMNP – North	Chamell Pluim	021 689 4441
Section Ranger	Chamell.Pluim@sanparks.org	
TMNP – North	Jaclyn Smith	021 422 2803
	jaclyn.smith@sanparks.org	

Yours faithfully,

Debbi Winterton, Science Liaison Officer; E-mail: deborah.winterton@sanparks.org

addo elephant
agulhas
augrabies
bontebok
camdeboo
golden gate highlands
karoo
kgalagadi transfrontier
knysna lake area
kruger
mapungubwe
marakele
mokala
mountain zebra
namaqua
table mountain
tankwa karoo
tsitsikamma
richtersveld
west coast
wilderness

643 Leyds Street
Muckleneuk
Pretoria

PO Box 787
Pretoria
0001

tel: 012 426 5000
fax: 012 343 0905

central reservations: 012 428 9111
reservations@parks.co.za
www.parks-sa.co.za

Figure A8: A copy of page two of the research study permit (for the years 2020 – 2021) within the vicinity of the Table Mountain National Park from the South African National Parks' Authorities.

To develop, expand, manage and promote a system of sustainable national parks that represent biodiversity and heritage assets, through innovation and best practice for the just and equitable benefit of current and future generations.			
Permit number: CRC/2021/024--2019/V1			
CAPE RESEARCH CENTRE P.O. Box 216, STEENBERG, 7947 Tel: +27 (0)21 713 7511; Fax: +27 (0)21 712 0131			
Research Permit: TABLE MOUNTAIN NATIONAL PARK		01 June 2021	
Dr. Marise Heyns - "Insect fauna and their pattern of succession on decomposing pig carcasses: Implications for Forensic Entomology in Western Cape, South Africa" <u>Co-workers:</u> Adeyemi Daniel Adetimehin, Wilmari Uys, Kyle Sieghard Kulenkampff,			
Division of forensic medicine and toxicology University of Cape Town, Observatory			
This permit is valid from 01 June 2021 until 31 December 2021. The approval is subject to the following conditions. The Park Management staff must be contacted prior to entry into the park (see list of staff members below). You are allowed access to the Northern section of the park (please see attached map for restricted areas).			
Standard Conditions: • The use of non-demarcated areas will lead to the disturbance of animals and eco-systems, trampling of vegetation and soil erosion and only the use of accepted pathways and areas is therefore permitted, UNLESS BY SPECIAL ARRANGEMENTS. PLEASE CONTACT THE PARK MANAGEMENT STAFF IF RESTRICTED AREAS NEED TO BE ACCESSED. • No damage shall be permitted to any natural vegetation, environment or property. • Animals may not be disturbed in any way. • Uncontrolled vehicle access and parking could cause damage to vegetation and soil erosion and therefore only the use of approved vehicles routes and parking areas is allowed. • Fires can cause loss of vegetation, soil erosion and life and therefore fires, and braai's are not permitted unless in dedicated braai areas. • Remove all rubbish and waste as it has an effect on the health of visitors, animals and plants. • Other visitors to the area and or neighbours may not be hindered in any way. • Pollution affects the health and safety of animals, plants, visitors and neighbours and is not permitted. • Excessive noise affects animals (e.g. birds nesting in the areas), visitors and or neighbours and is not permitted. • Your permit and identification must be retained and kept on your person at all times, and produced on request. • The areas under the control of SANParks are used entirely at your own risk. South African National Parks, its Board, directors, employees and agents are not liable for any loss or damage to the property or possession of any guest or participant (or accompanying minor) whether such damage is caused by the negligent act or omission of South African National Parks; arising from death or any bodily injuries of whatsoever nature sustained by a guest or participant (or accompanying minor) whether such injuries are caused by the negligent act or omission by South African National Parks, and/or by the defective functioning of any apparatus. The guest or participant and/or his/her/their estate hereby indemnifies South African National Parks against any claim, action, judgment, costs and/or expenses which may be made against South African National Parks and as may in any way be related to the above. The onus lies with the company or applicant to ensure that they are adequately insured.		addo elephant agulhas augrabies bontebok camdeboo golden gate highlands karoo kgalagadi transfrontier knysna lake area kruger mapungubwe marakele mokala mountain zebra namaqua table mountain tankwa karoo tsitsikamma richtersveld west coast wilderness	
643 Leyds Street Muckleneuk Pretoria	PO Box 787 Pretoria 0001	tel: 012 426 5000 fax: 012 343 0905	central reservations: 012 428 9111 reservations@parks.co.za www.parks-sa.co.za

Figure A9: A copy of page one of the research study permit (for the year June 2021 – December 2021) within the vicinity of the Table Mountain National Park from the South African National Parks' Authorities.

To develop, expand, manage and promote a system of sustainable national parks that represent biodiversity and heritage assets, through innovation and best practice for the just and equitable benefit of current and future generations.



South African
NATIONAL PARKS

- Please note that you (your delegates, staff etc) are subject to the conditions set in terms of Section 86(1) of the National Environmental Management Act (107 of 1998) and the National Environmental Act: Protected Areas Act (Act 57 of 2003) for the duration of your stay in the National Park. Your attention is specifically drawn to sections 64(1) (a), (b) & (c) which refers to penalties in terms of the Act.
- SANParks staff's instructions, notices, regulations and signs must be complied with.
- The activity shall be restricted to the area applied for.
- Gate and operating times to be complied with.
- NO PETS ALLOWED

Special Conditions:

- The researches may work in the area of the park surrounding UCT.
- Every month, two stillborn pig carcasses of similar weights will be placed individually in purpose designed fly traps a minimum of 30 meters apart. Thereafter, pitfall traps will be placed at four different points (north, south, east, west) around each of the fly trap at a minimum of 1 meter.
- Pig carcasses must be appropriately hidden and camouflaged in brown netting and vegetation.
- Adult flying and ground-crawling insects attracted to the stillborn pig carcasses will be collected.

Area	Park Management Staff	Telephone Number:
TMNP – North Section Ranger	Chamell Pluim Chamell.Pluim@sanparks.org	021 689 4441
TMNP – North	Jaclyn Smith jaclyn.smith@sanparks.org	021 422 2803

Yours faithfully,

Debbi Winterton, Science Liaison Officer; E-mail: deborah.winterton@sanparks.org

addo elephant
agulhas
augrabies
bontebok
camdeboo
golden gate highlands
karoo
kgalagadi transfrontier
knysna lake area
kruger
mapungubwe
marakele
mokala
mountain zebra
namaqua
table mountain
tankwa karoo
tsitsikamma
richtersveld
west coast
wilderness

643 Leyds Street
Muckleneuk
Pretoria

PO Box 787
Pretoria
0001

tel: 012 426 5000
fax: 012 343 0905

central reservations: 012 428 9111
reservations@parks.co.za
www.parks-sa.co.za

Figure A10: A copy of page two of the research study permit (for the year June 2021 – December 2021) within the vicinity of the Table Mountain National Park from the South African National Parks' Authorities.

To develop, expand, manage and promote a system of sustainable national parks that represent biodiversity and heritage assets, through innovation and best practice for the just and equitable benefit of current and future generations.			
Permit number: CRC/2022 - 2023/024--2019/V1			
CAPE RESEARCH CENTRE P.O. Box 216, STEENBERG, 7947 Tel: +27 (0)21 713 7511; Fax: +27 (0)21 712 0131			
Research Permit: TABLE MOUNTAIN NATIONAL PARK		15 March 2022	
Dr. Marise Heyns - "Insect succession and changes in the soil pH and electrical conductivity associated with decomposing pig carcasses on the Table Mountain National Park of the Western Cape Province of South Africa"			
<u>Co-workers:</u> Adeyemi Daniel Adetimehin, Wilmari Uys, Kyle Sieghard Kulenkampff,			
Division of forensic medicine and toxicology University of Cape Town, Observatory			
This permit is valid from 15 March 2022 until 31 December 2023. The approval is subject to the following conditions. The Park Management staff must be contacted prior to entry into the park (see list of staff members below). You are allowed access to the Northern section of the park (please see attached map for restricted areas).		addo elephant agulhas augrabies bontebok camdeboo golden gate highlands karoo kgalagadi transfrontier knysna lake area kruger mapungubwe marakele mokala mountain zebra namaqua table mountain tankwa karoo tsitsikamma richtersveld west coast wilderness	
Standard Conditions: • The use of non-demarcated areas will lead to the disturbance of animals and eco-systems, trampling of vegetation and soil erosion and only the use of accepted pathways and areas is therefore permitted, UNLESS BY SPECIAL ARRANGEMENTS. PLEASE CONTACT THE PARK MANAGEMENT STAFF IF RESTRICTED AREAS NEED TO BE ACCESSED. • No damage shall be permitted to any natural vegetation, environment or property. • Animals may not be disturbed in any way. • Uncontrolled vehicle access and parking could cause damage to vegetation and soil erosion and therefore only the use of approved vehicles routes and parking areas is allowed. • Fires can cause loss of vegetation, soil erosion and life and therefore fires, and braai's are not permitted unless in dedicated braai areas. • Remove all rubbish and waste as it has an effect on the health of visitors, animals and plants. • Other visitors to the area and or neighbours may not be hindered in any way. • Pollution affects the health and safety of animals, plants, visitors and neighbours and is not permitted. • Excessive noise affects animals (e.g. birds nesting in the areas), visitors and or neighbours and is not permitted. • Your permit and identification must be retained and kept on your person at all times, and produced on request. • The areas under the control of SANParks are used entirely at your own risk. South African National Parks, its Board, directors, employees and agents are not liable for any loss or damage to the property or possession of any guest or participant (or accompanying minor) whether such damage is caused by the negligent act or omission of South African National Parks; arising from death or any bodily injuries of whatsoever nature sustained by a guest or participant (or accompanying minor) whether such injuries are caused by the negligent act or omission by South African National Parks, and/or by the defective functioning of any apparatus. The guest or participant and/or his/her/their estate hereby indemnifies South African National Parks against any claim, action, judgment, costs and/or expenses which may be made against South African National Parks and as may in any way be related to the above. The onus lies with the company or applicant to ensure that they are adequately insured.			
643 Leyds Street Muckleneuk Pretoria	PO Box 787 Pretoria 0001	tel: 012 426 5000 fax: 012 343 0905	central reservations: 012 428 9111 reservations@parks.co.za www.parks-sa.co.za

Figure A11: A copy of page one of the research study permit (for the years 2022 – 2023) within the vicinity of the Table Mountain National Park from the South African National Parks' Authorities.

To develop, expand, manage and promote a system of sustainable national parks that represent biodiversity and heritage assets, through innovation and best practice for the just and equitable benefit of current and future generations.



- Please note that you (your delegates, staff etc) are subject to the conditions set in terms of Section 86(1) of the National Environmental Management Act (107 of 1998) and the National Environmental Act: Protected Areas Act (Act 57 of 2003) for the duration of your stay in the National Park. Your attention is specifically drawn to sections 64(1) (a), (b) & (c) which refers to penalties in terms of the Act.
- SANParks staff's instructions, notices, regulations and signs must be complied with.
- The activity shall be restricted to the area applied for.
- Gate and operating times to be complied with.
- NO PETS ALLOWED

Special Conditions:

- The researches may work in the area of the park surrounding UCT.
- Two adult pig carcasses may be placed in the field, one during the warmer months and one during the cold months.
- Pig carcasses must be appropriately hidden and camouflaged in brown netting and vegetation.
- Adult flying and ground-crawling insects attracted to the pig carcasses will be collected by means of pitfall traps and fly traps..

Area	Park Management Staff	Telephone Number:
TMNP – North Section Ranger	vacant	021 689 4441
TMNP – North	Mathabatha Matjila mathabatha.matjila@sanparks.org	021 422 2803

Yours faithfully,

Debbi Winterton, Science Liaison Officer; E-mail: deborah.winterton@sanparks.org

addo elephant
agulhas
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golden gate highlands
karoo
kgalagadi transfrontier
knysna lake area
kruger
mapungubwe
marakele
mokala
mountain zebra
namaqua
table mountain
tankwa karoo
tsitsikamma
richtersveld
west coast
wilderness

643 Leyds Street
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Pretoria

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0001

tel: 012 426 5000
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reservations@parks.co.za
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Figure A12: A copy of page two of the research study permit (for the years 2022 – 2023) within the vicinity of the Table Mountain National Park from the South African National Parks' Authorities.

Appendix B: Decomposition stages of the pig carcasses, insect diversity, abundance, and occurrence matrices for the monthly neonate pig trials



Figure B1: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the mid-spring neonate pig trial.



Figure B2: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the late spring neonate pig trial.

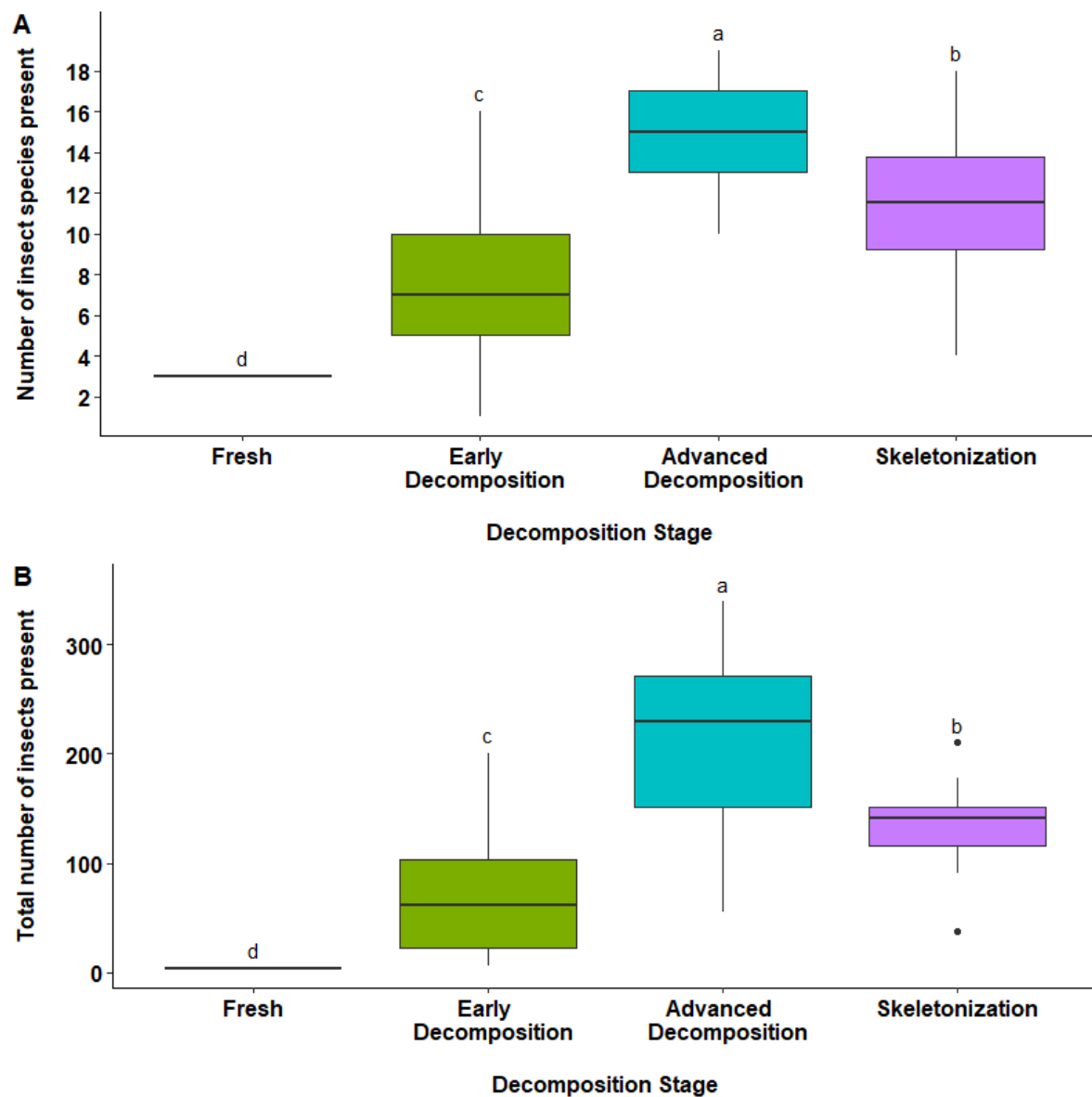


Figure B3: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the early spring neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

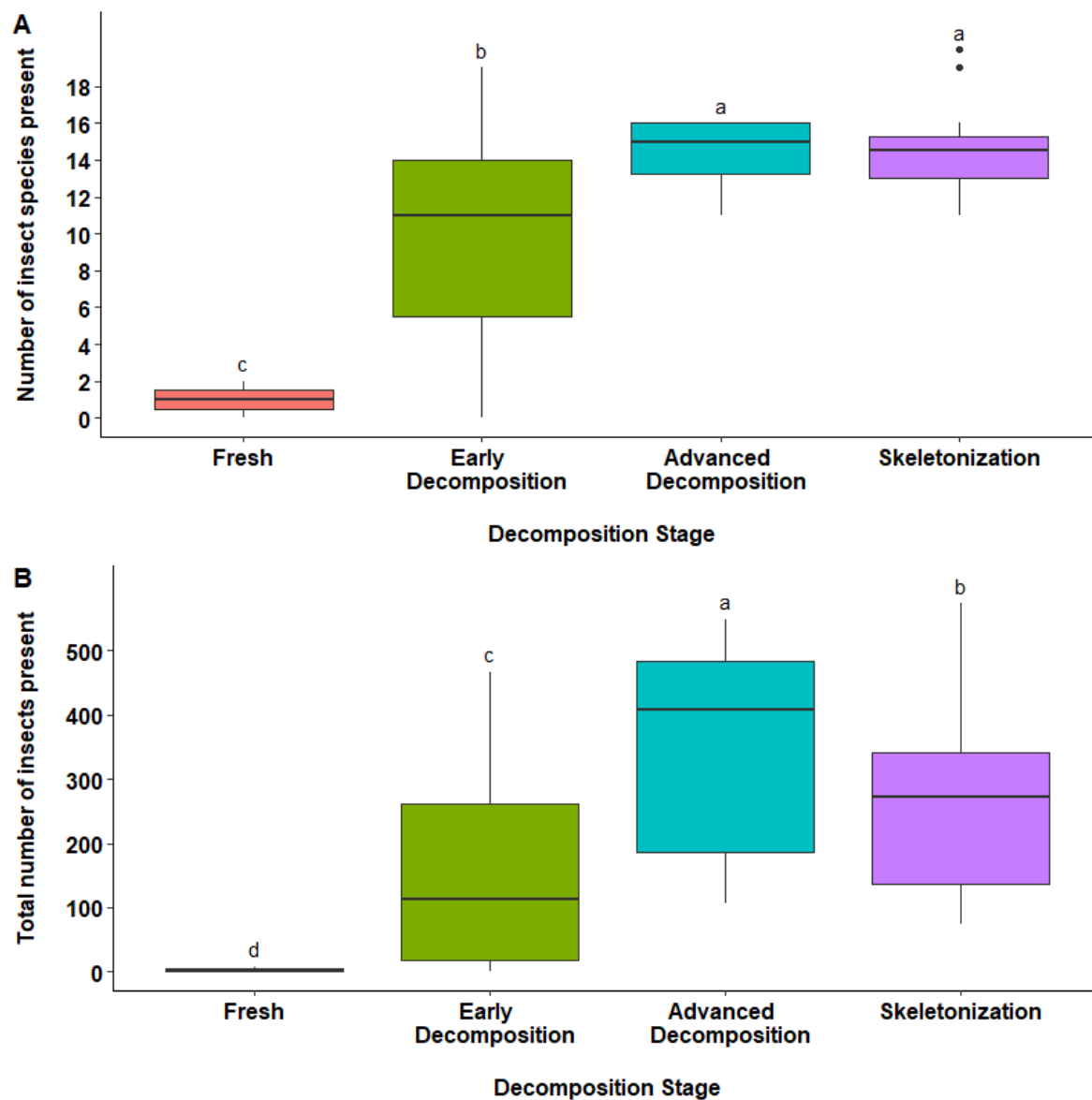


Figure B4: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the mid-spring neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

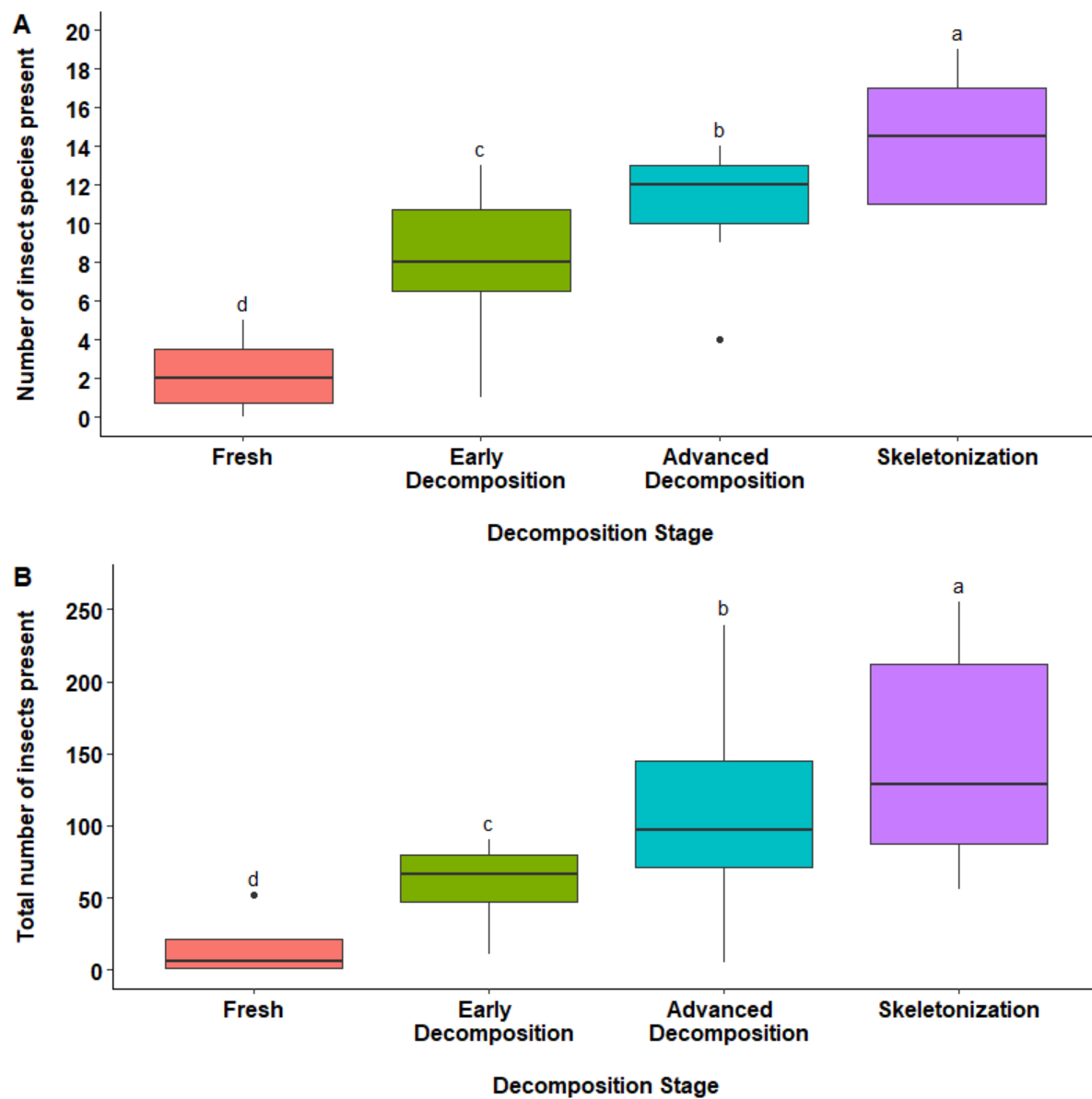


Figure B5: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the late spring neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

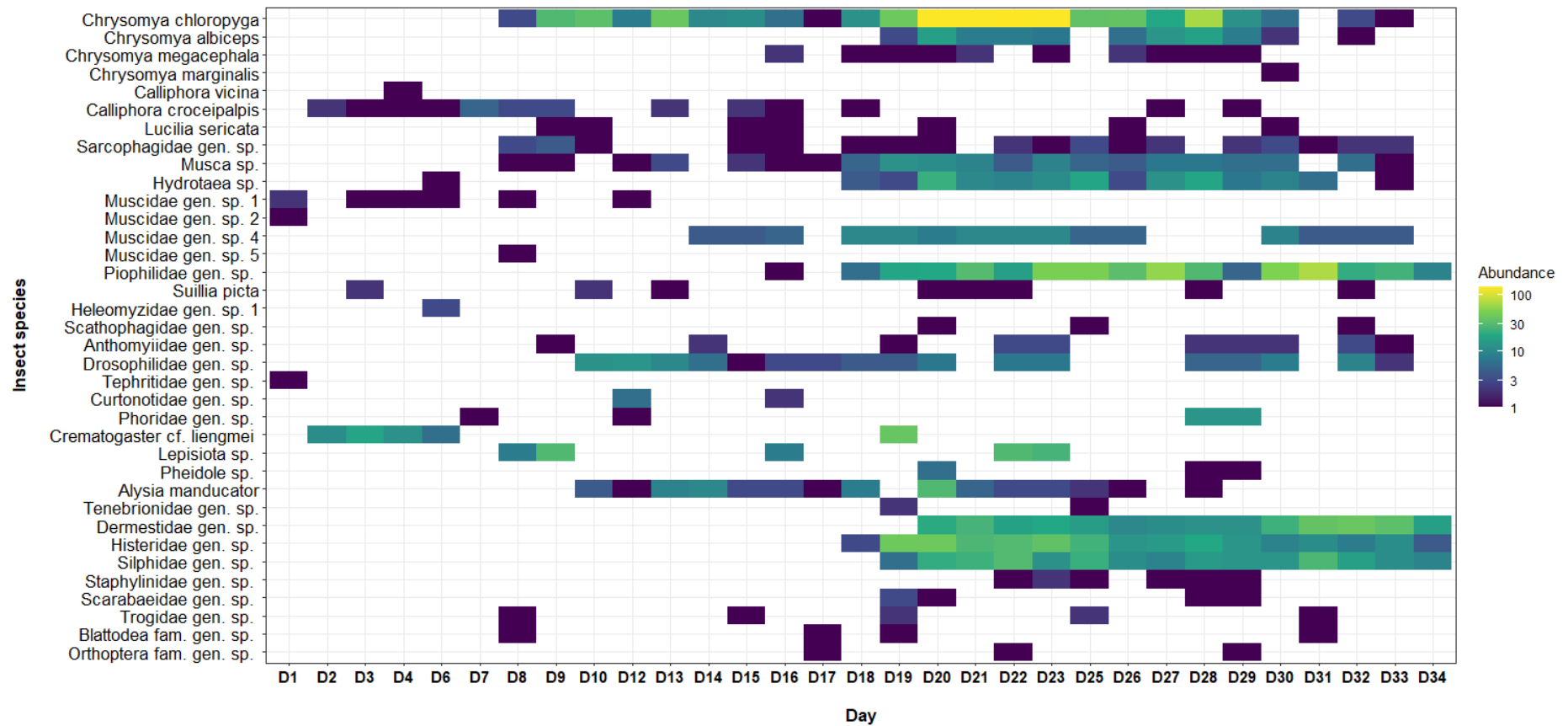


Figure B6: Daily abundance of insect species on pig carcass 1 in the early spring neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D22; Advanced decomposition: D23 – D26; Skeletonization: D27 – D34. Eggs and L1 larvae of *Ch. chloropyga* were recorded on Day 9 and Day 10 respectively; Eggs and L1 larvae of *Ca. vicina* were recorded on Day 4 and Day 7 respectively; Silphidae gen. sp. larvae were recorded on Day 27 until the end of the trial.

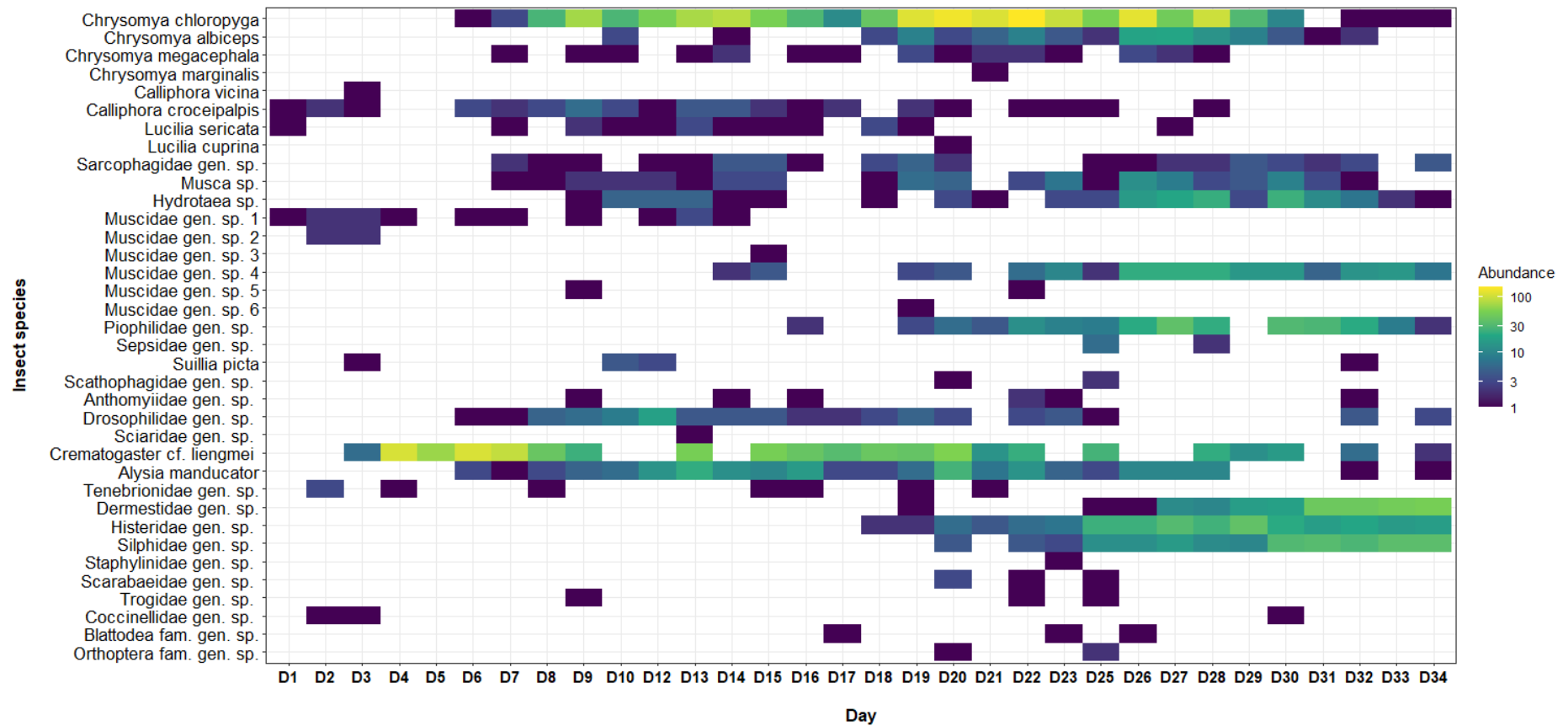


Figure B7: Daily abundance of insect species on pig carcass 2 in the early spring neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D19; Advanced decomposition: D20 – D28; Skeletonization: D29 – D34. Eggs and L1 larvae of *Ca. croceipalpis* were recorded on Day 3 and Day 7 respectively; L1 larvae of *Sarcophagidae* gen. sp. were recorded on Day 8; *Silphidae* gen. sp. larvae were recorded on Day 26 until the end of the trial.

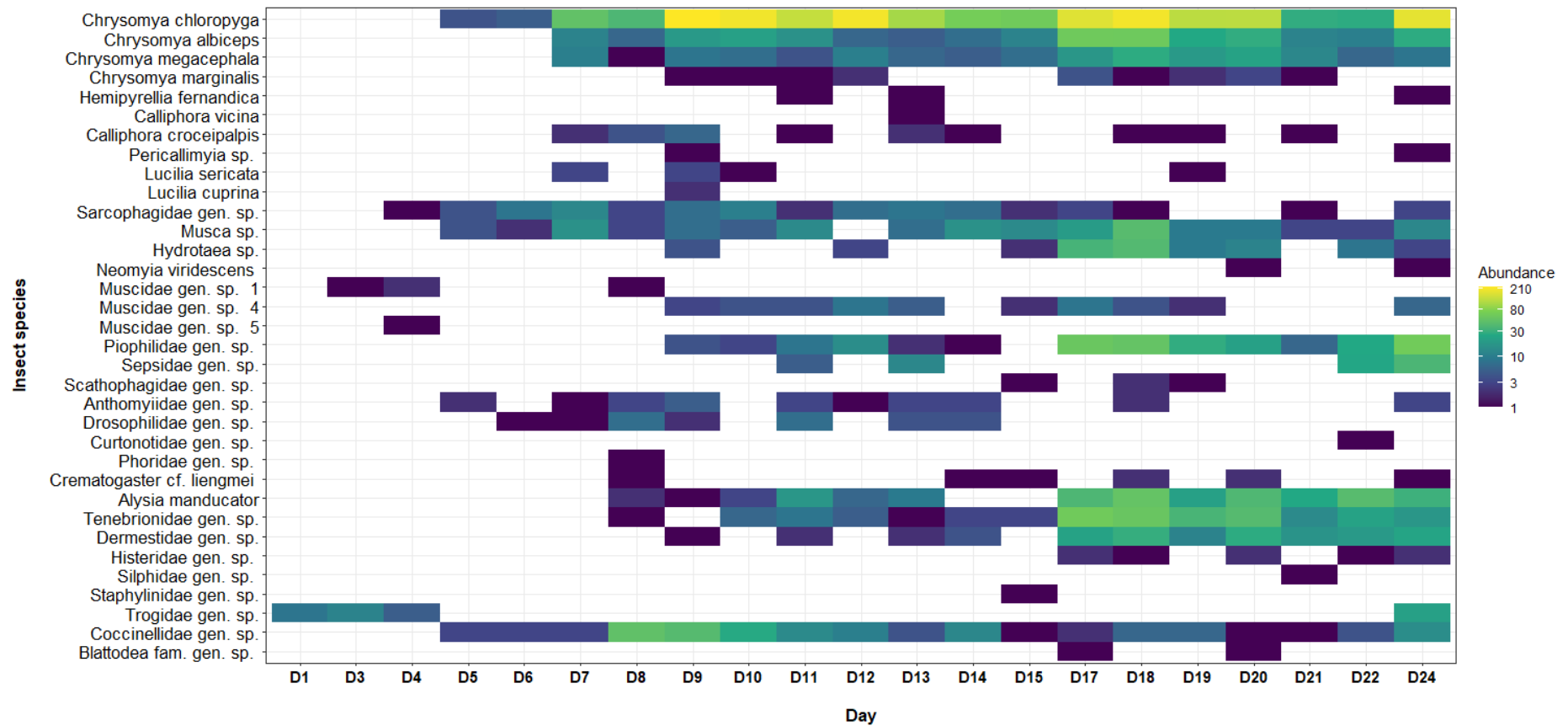


Figure B8: Daily abundance of insect species on pig carcass 1 in the mid-spring neonate pig trial. Fresh stage: D1; Early decomposition: D2 – D13; Advanced decomposition: D14 – D17; Skeletonization: D18 – D24. Eggs and L1 larvae of *Ch. chloropyga* were recorded on Day 8 and Day 9 respectively; *Dermestidae* gen. sp. and *Silphidae* gen. sp. larvae were both recorded on Day 22 until the end of the trial.

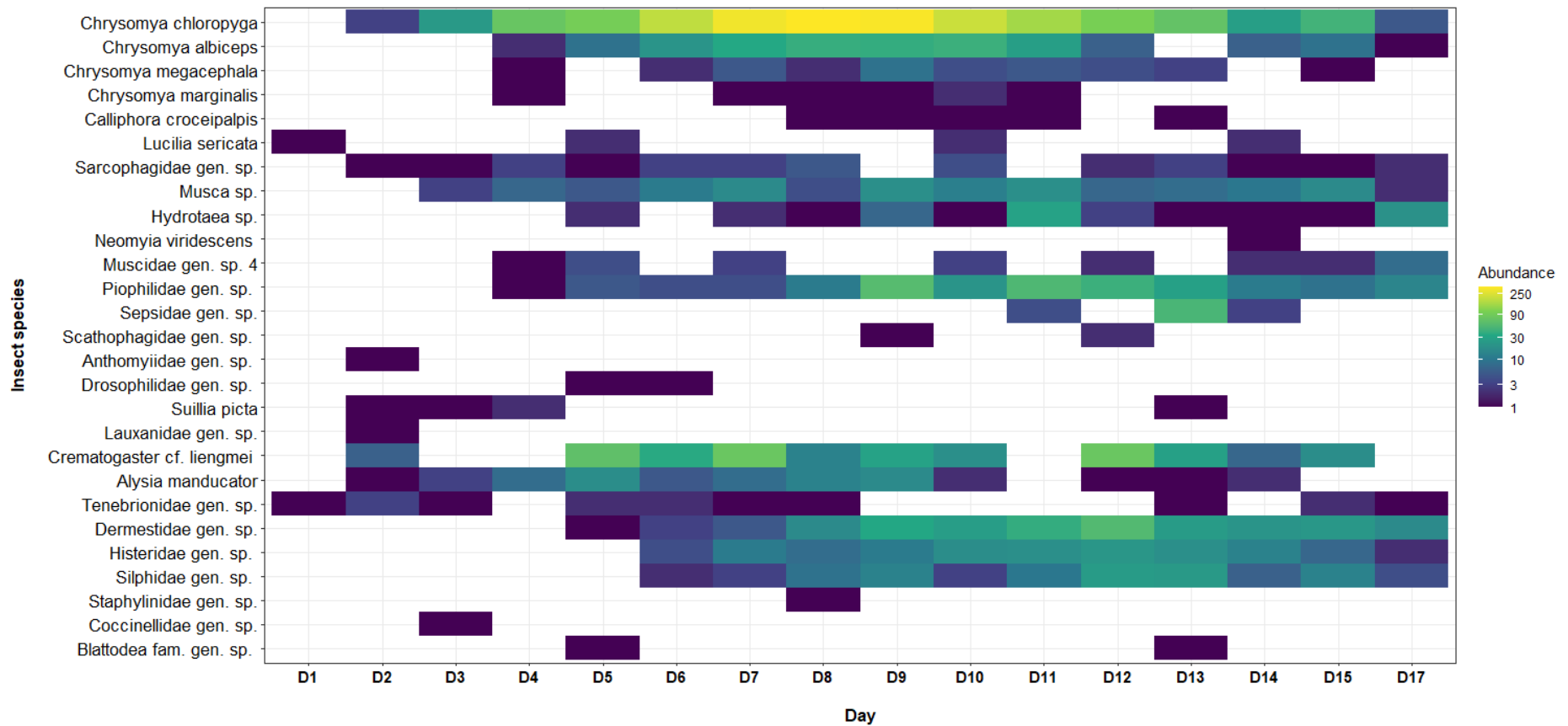


Figure B9: Daily abundance of insect species on pig carcass 2 in the mid-spring neonate pig trial. Fresh stage: D1; Early decomposition: D2 – D7; Advanced decomposition: D8 – D10; Skeletonization: D11 – D17. Eggs and L1 larvae of *Ch. chloropyga* were recorded on Day 2 and Day 4 respectively; L1 larvae of *Sarcophagidae* gen. sp. were recorded on Day 5; *Silphidae* gen. sp. larvae were recorded on Day 17.

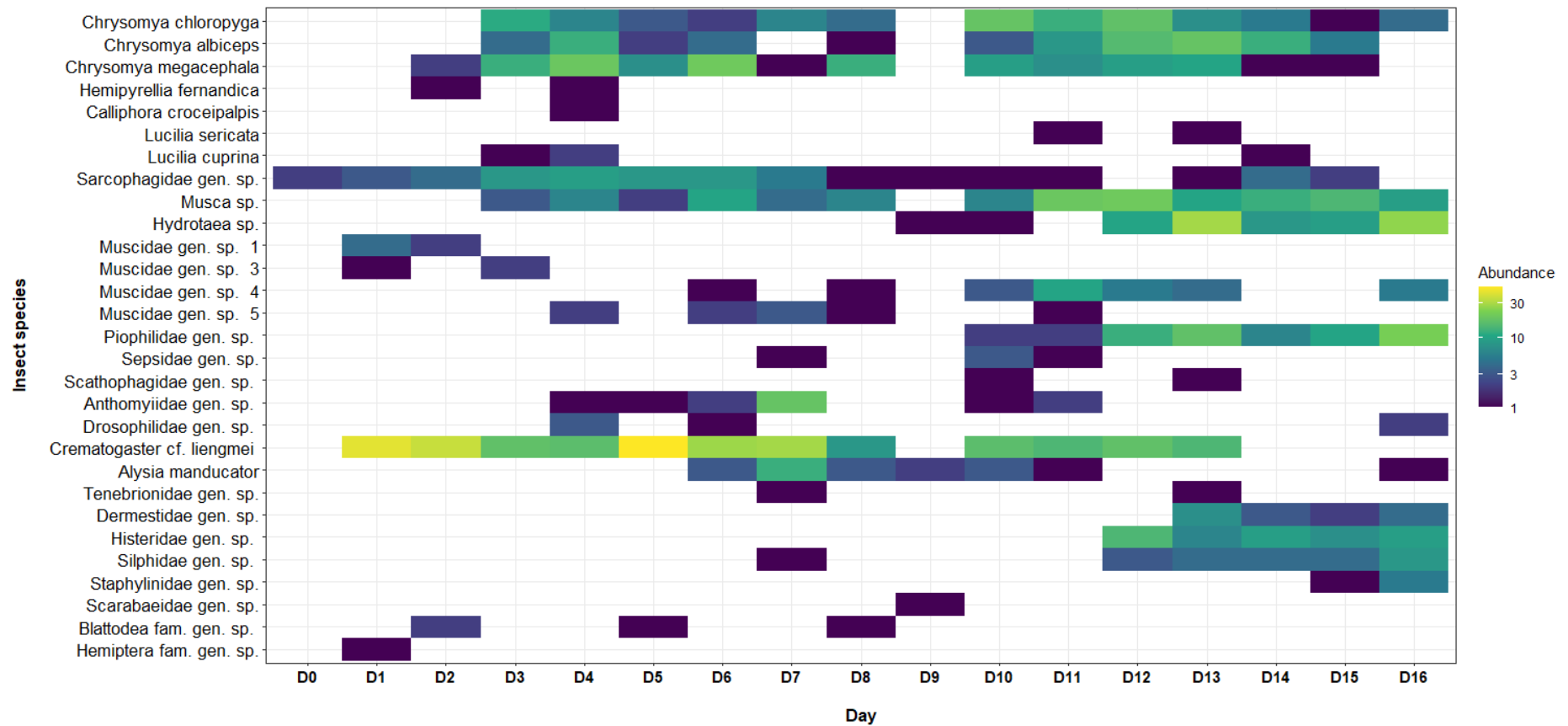


Figure B10: Daily abundance of insect species on pig carcass 1 in the late spring neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D6; Advanced decomposition: D7 – D12; Skeletonization: D13 – D16. L1 larvae of Sarcophagidae gen. sp. were recorded on Day 6.

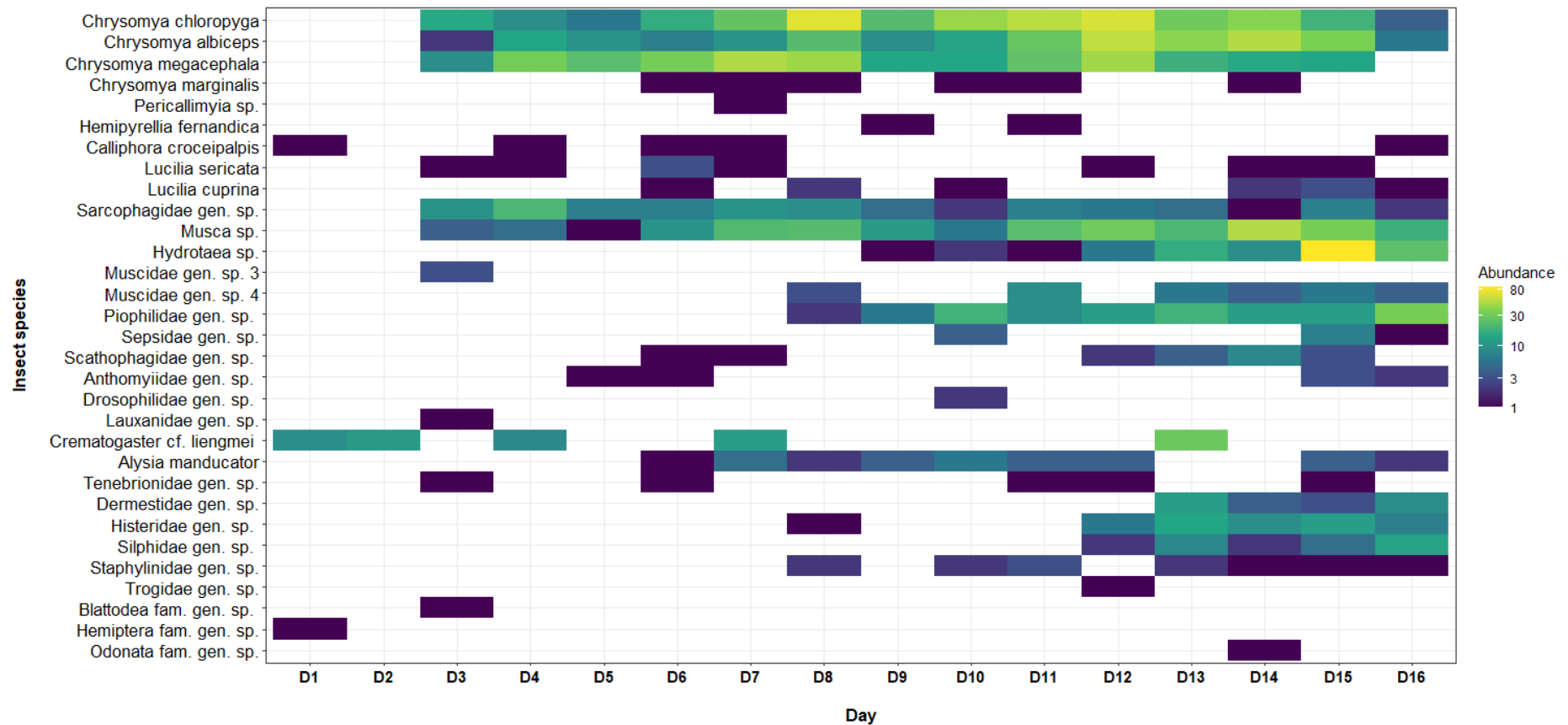


Figure B11: Daily abundance of insect species on pig carcass 2 in the late spring neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D6; Advanced decomposition: D7 – D12; Skeletonization: D13 – D16. Eggs, L1 and L2 larvae of *Ch. chloropyga* were recorded on Days 8/11 and Day 12 respectively; L1 larvae of *Sarcophagidae* gen. sp. were recorded on Day 4.



Figure B12: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the mid-summer neonate pig trial.

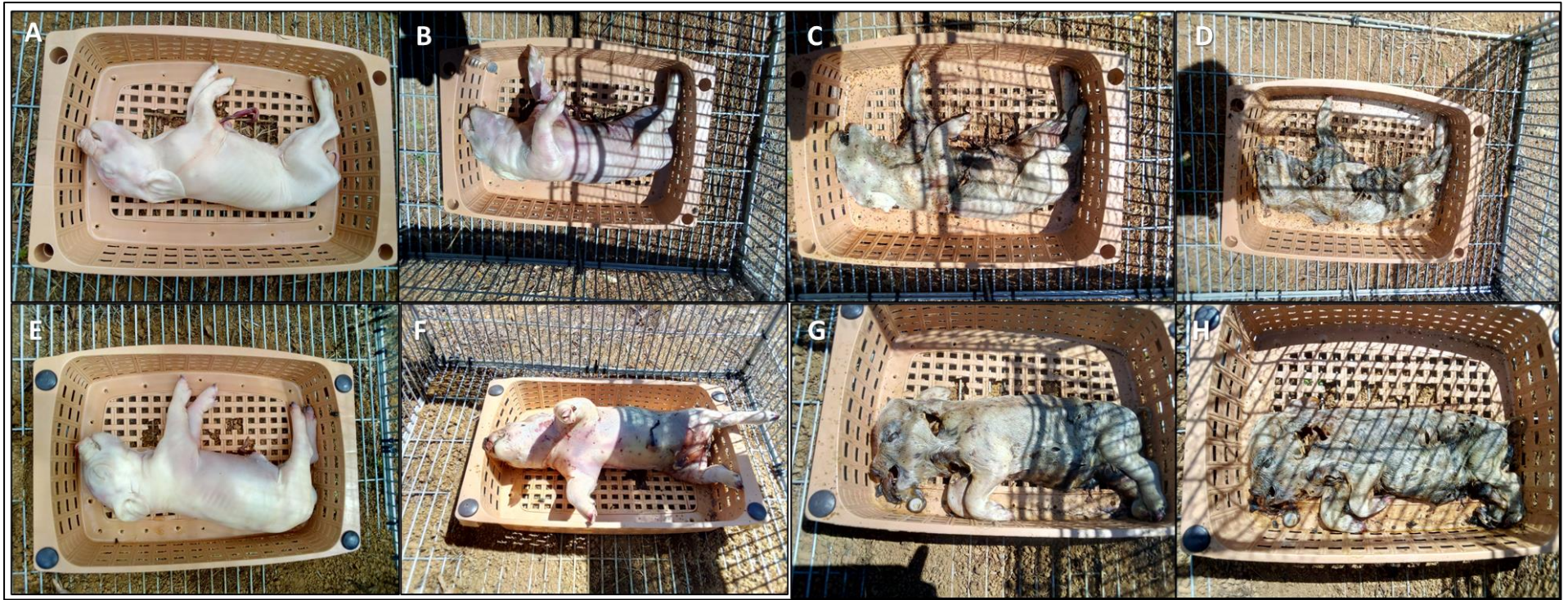


Figure B13: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the late summer neonate pig trial.

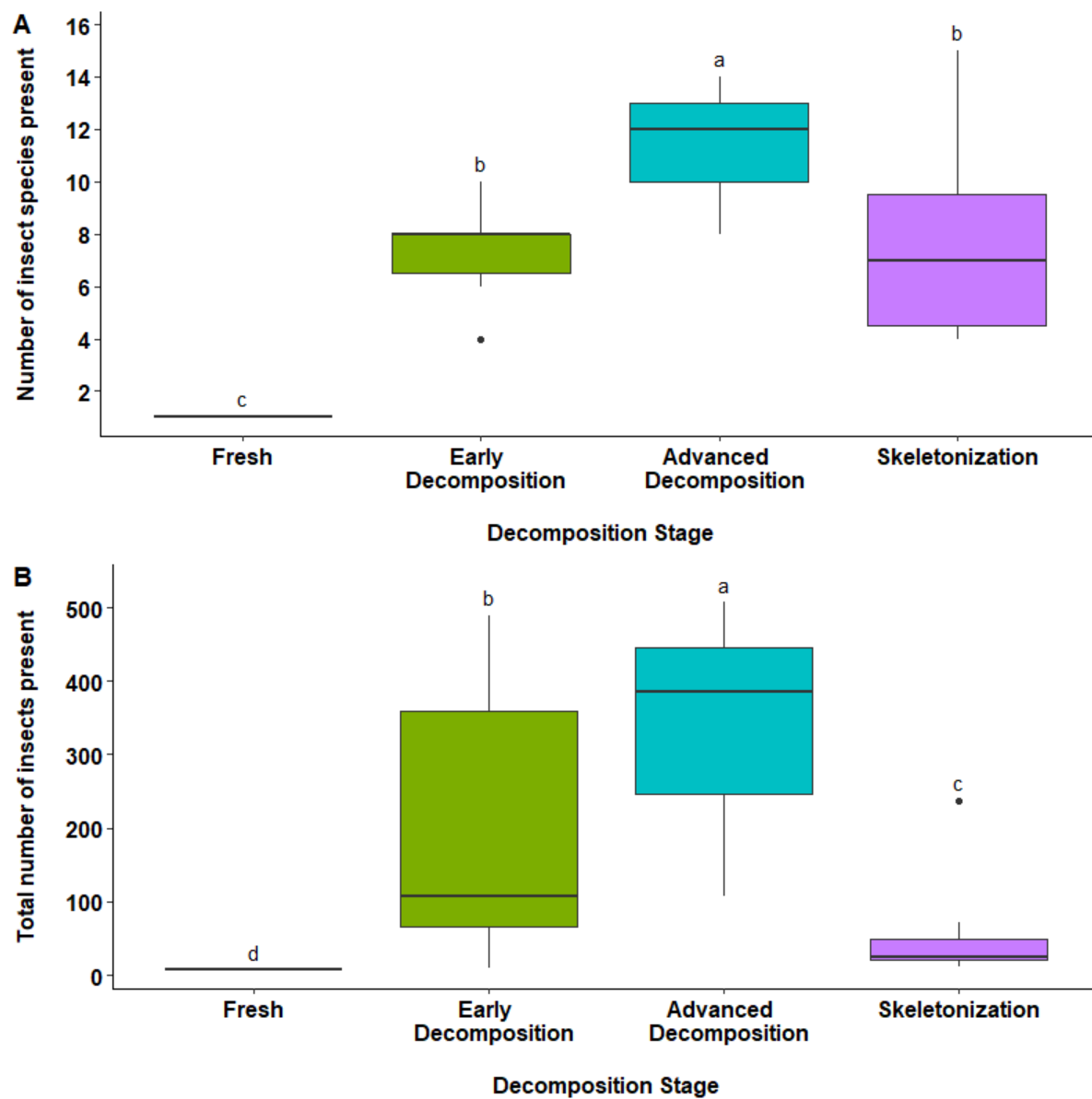


Figure B14: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the early summer neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

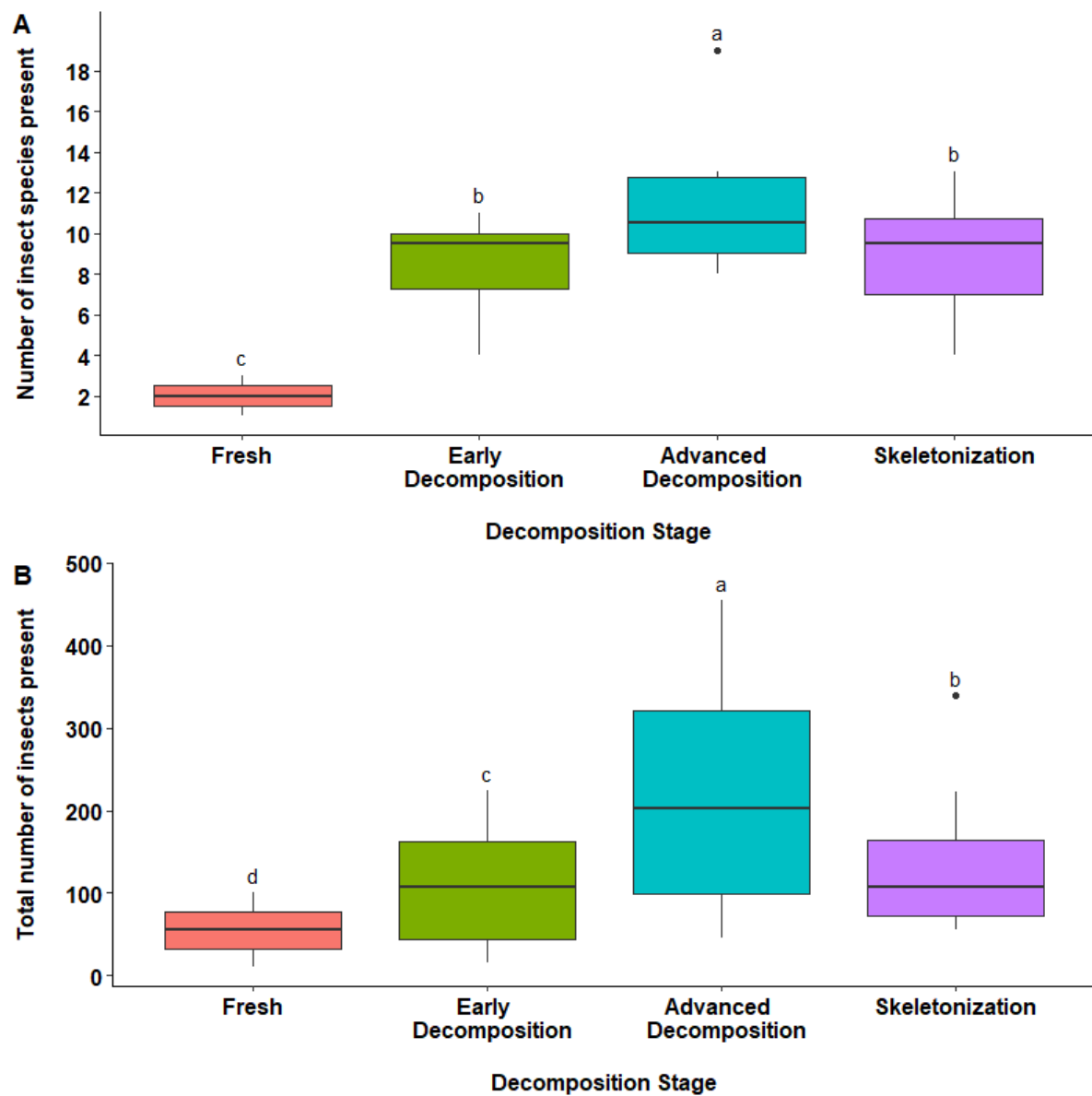


Figure B15: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the mid-summer neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

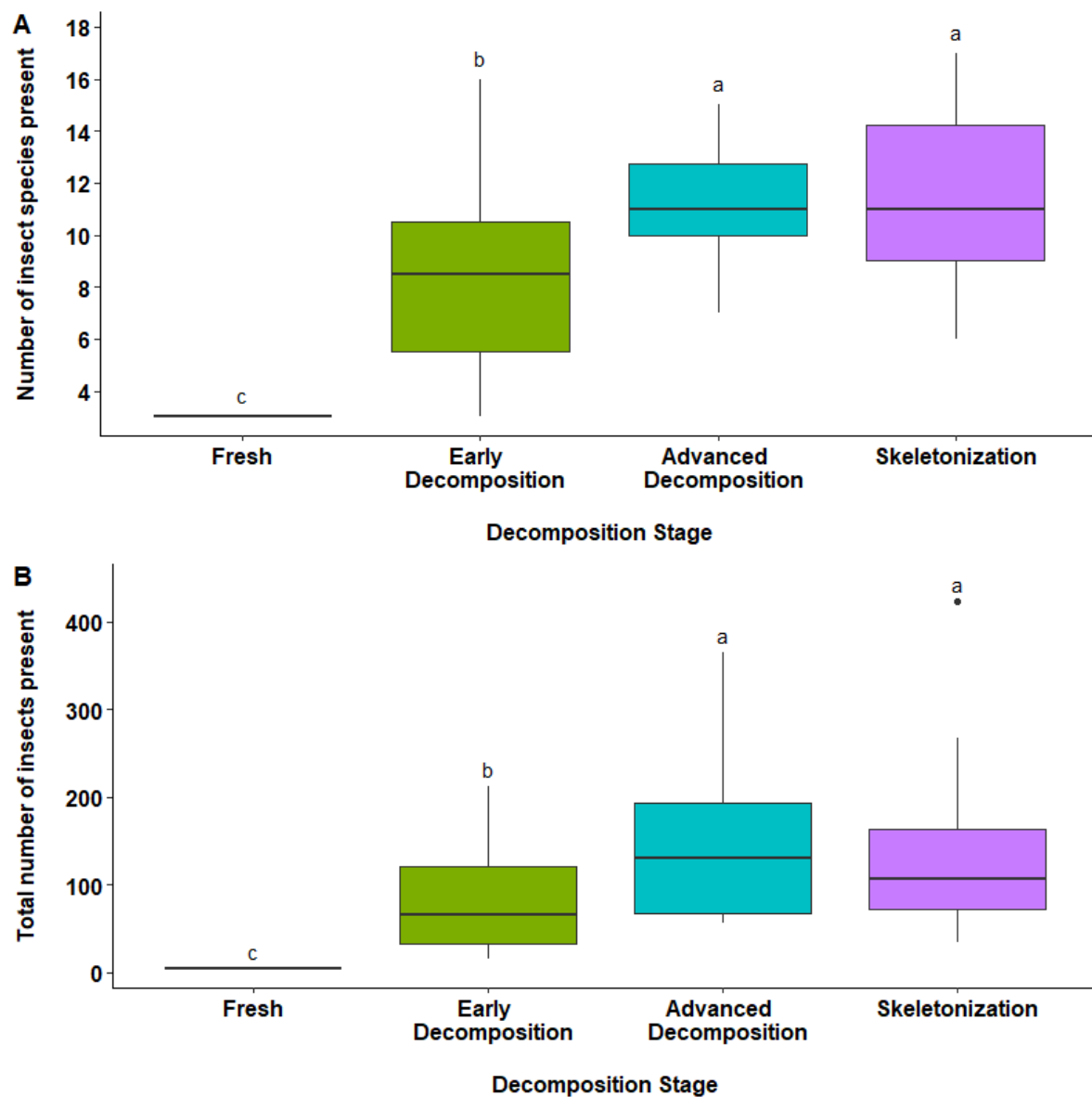


Figure B16: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the late summer neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

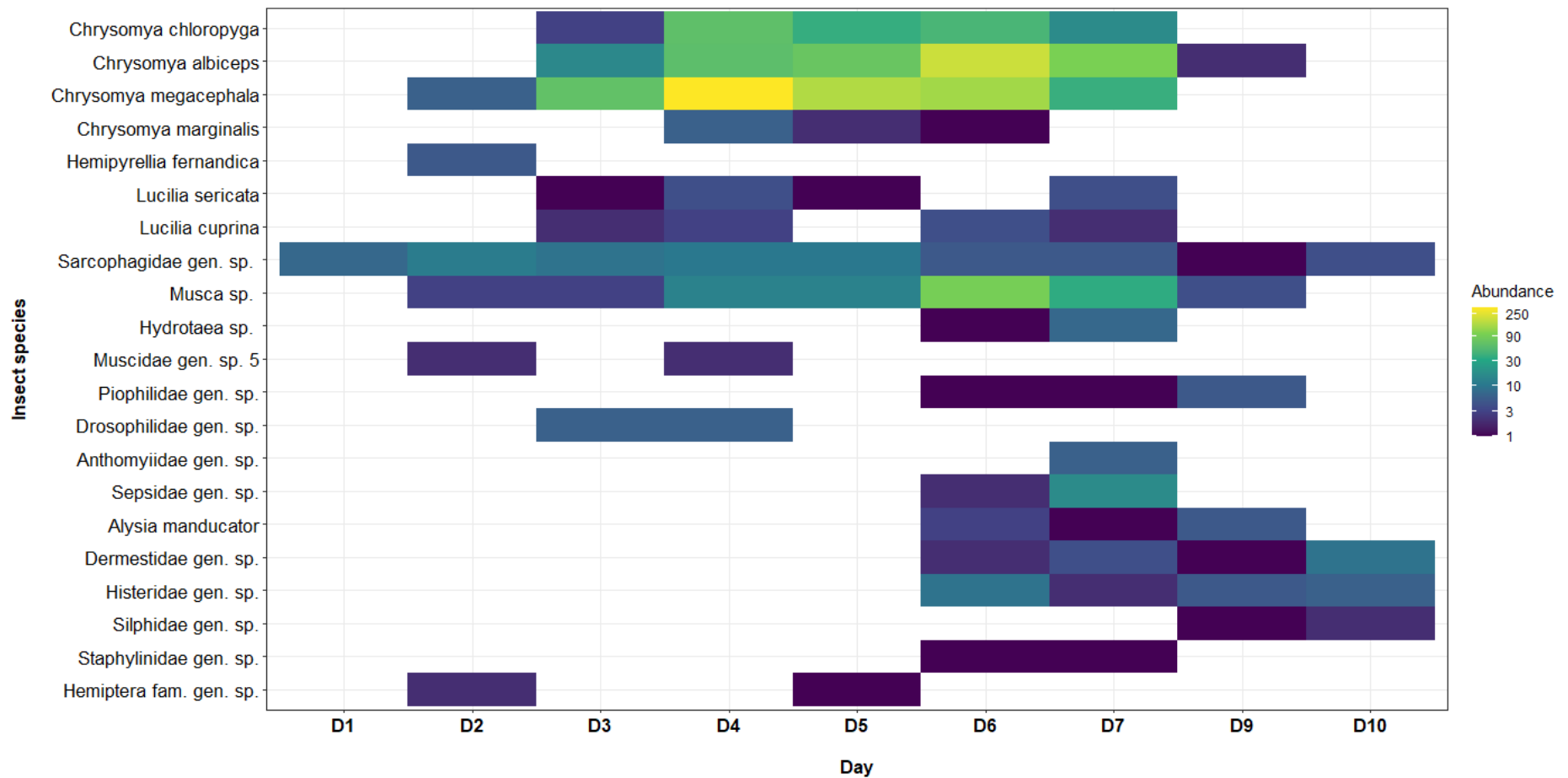


Figure B17: Daily abundance of insect species on pig carcass 1 in the early summer neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D5; Advanced decomposition: D6; Skeletonization: D7 – D10. L1 larvae of *Ch. albiceps*, *Ch. chloropyga*, and *Ch. marginalis* respectively, were recorded on Day 4; L1 and L2 larvae of *Sarcophagidae gen. sp.* were recorded on Day 2.

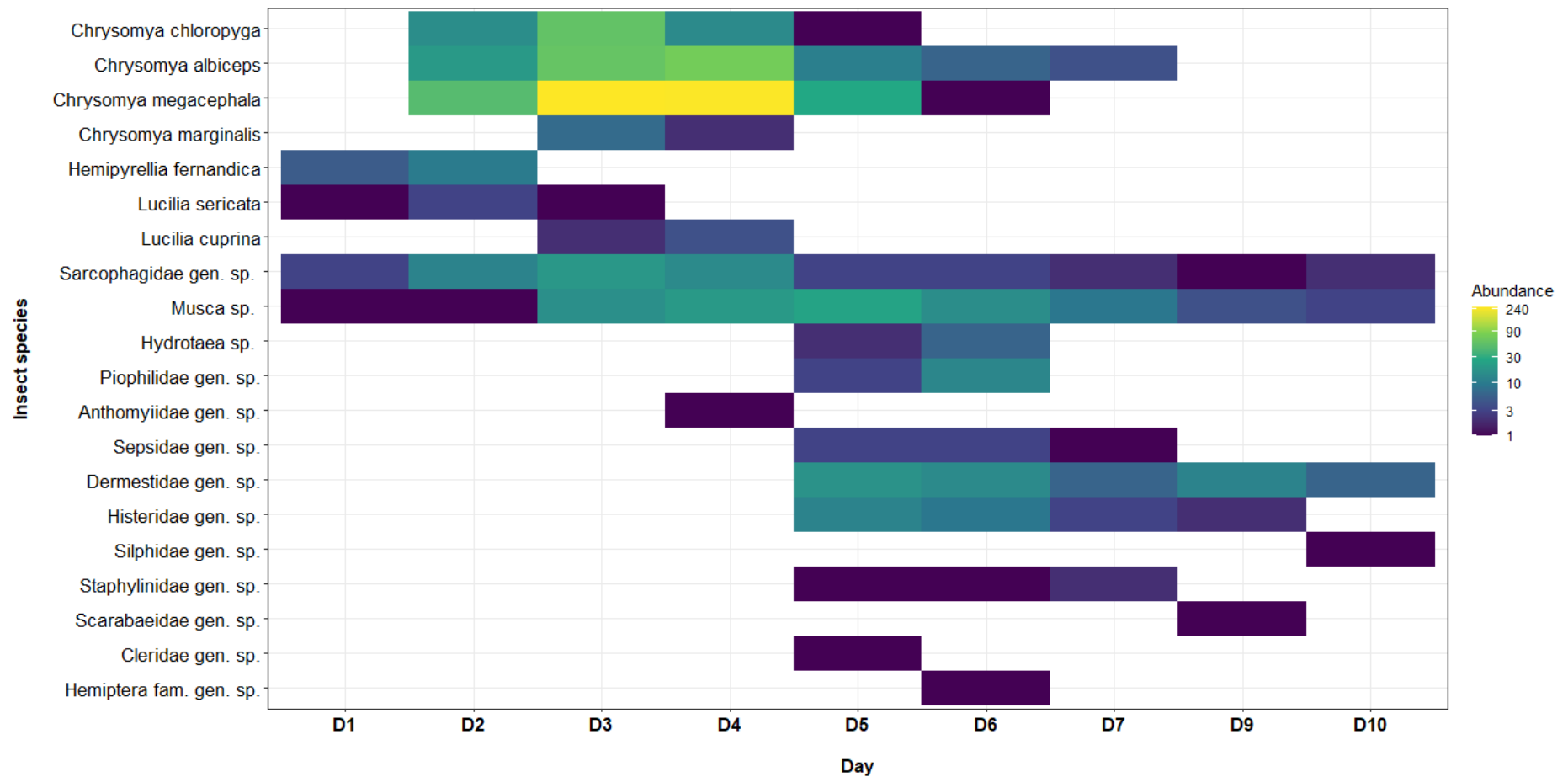


Figure B18: Daily abundance of insect species on pig carcass 2 in the early summer neonate pig trial. Fresh stage: D0; Early decomposition: D1 – D3; Advanced decomposition: D4 – D5; Skeletonization: D6 – D10. L1 larvae of *Sarcophagidae* gen. sp. were recorded on Day 1.

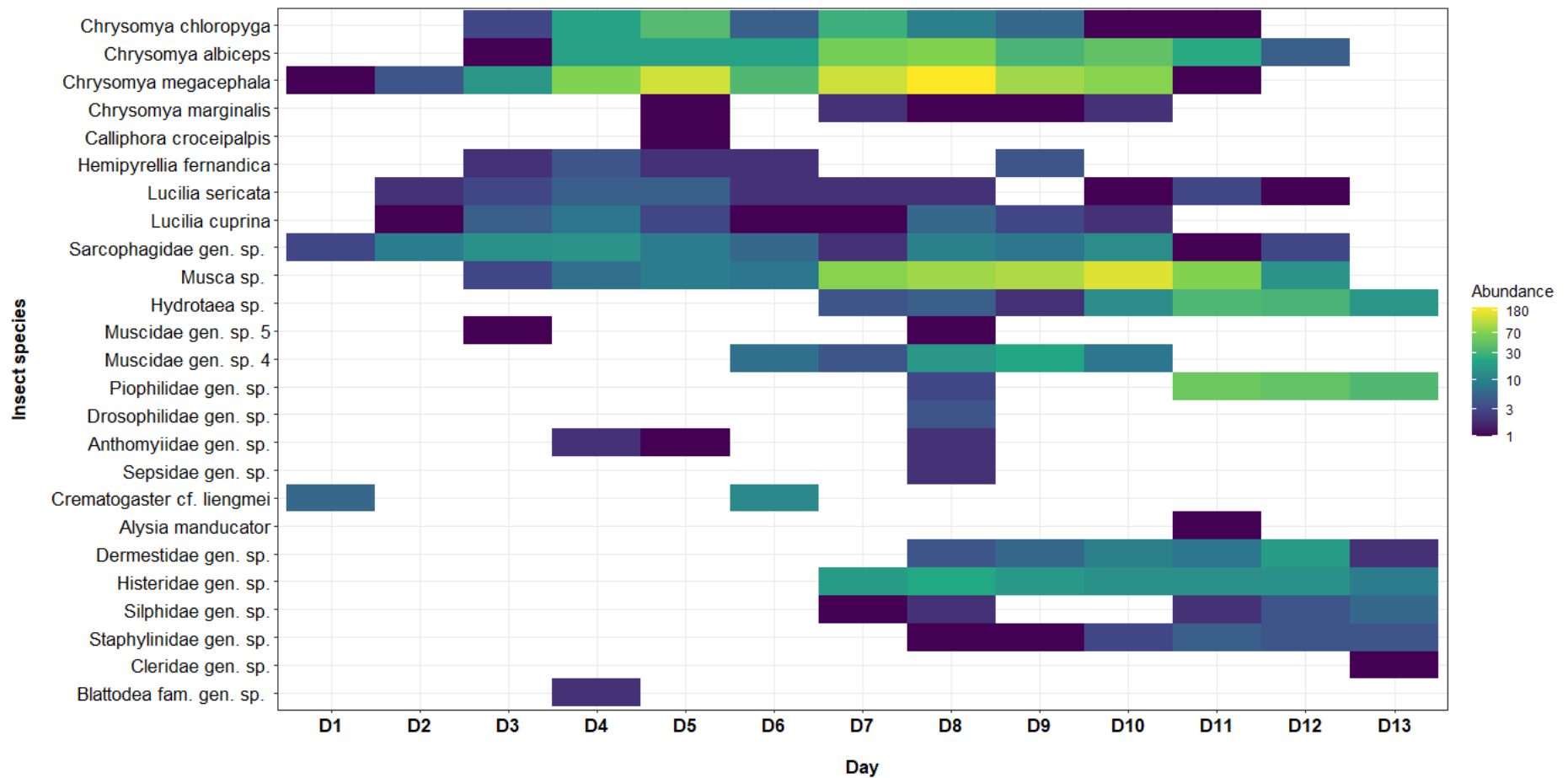


Figure B19: Daily abundance of insect species on pig carcass 1 in the mid-summer neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D6; Advanced decomposition: D7 – D9; Skeletonization: D10 – D13. Eggs, L1 and L2 larvae of *Ch. chloropyga* were seen on Day 5 and 6. Eggs, and L1 and L2 larvae of *Ch. albiceps* were recorded on Day 6 and 7 respectively. L1 and L2 larvae of *Ch. megacephala* were recorded on Day 6; L2 larvae of *Sarcophagidae gen. sp.* were recorded on Day 2.

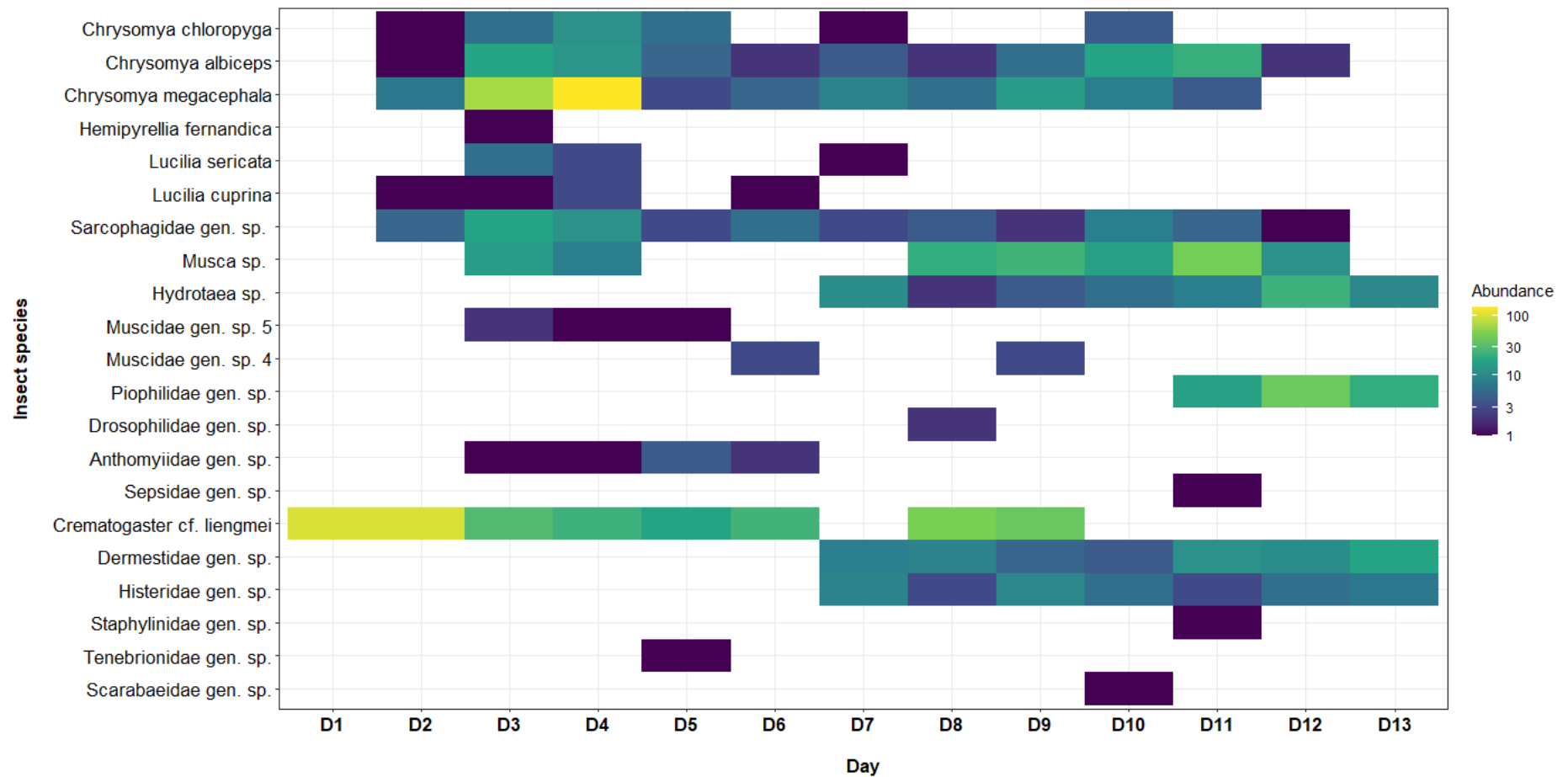


Figure B20: Daily abundance of insect species on pig carcass 2 in the mid-summer neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D6; Advanced decomposition: D7 – D9; Skeletonization: D10 – D13. L1 larvae of *Sarcophagidae* gen. sp. were recorded on Day 3.

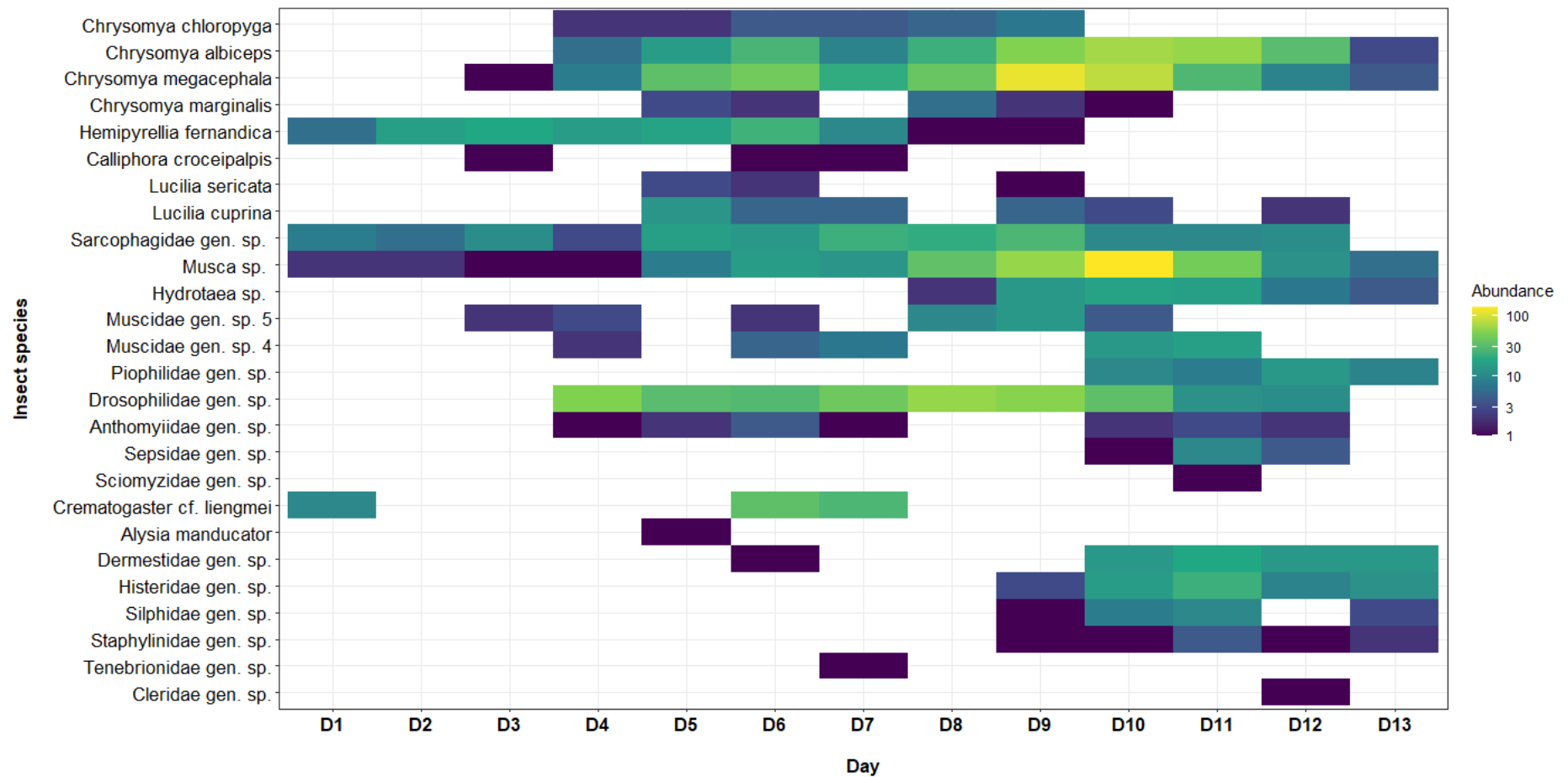


Figure B21: Daily abundance of insect species on pig carcass 1 in the late summer neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D6; Advanced decomposition: D7 – D9; Skeletonization: D10 – D13. Eggs of *Ch. megacephala* and *Ch. chloropyga* were both recorded on Day 8; L2 larvae of *Sarcophagidae* gen. sp. were recorded on Day 5.

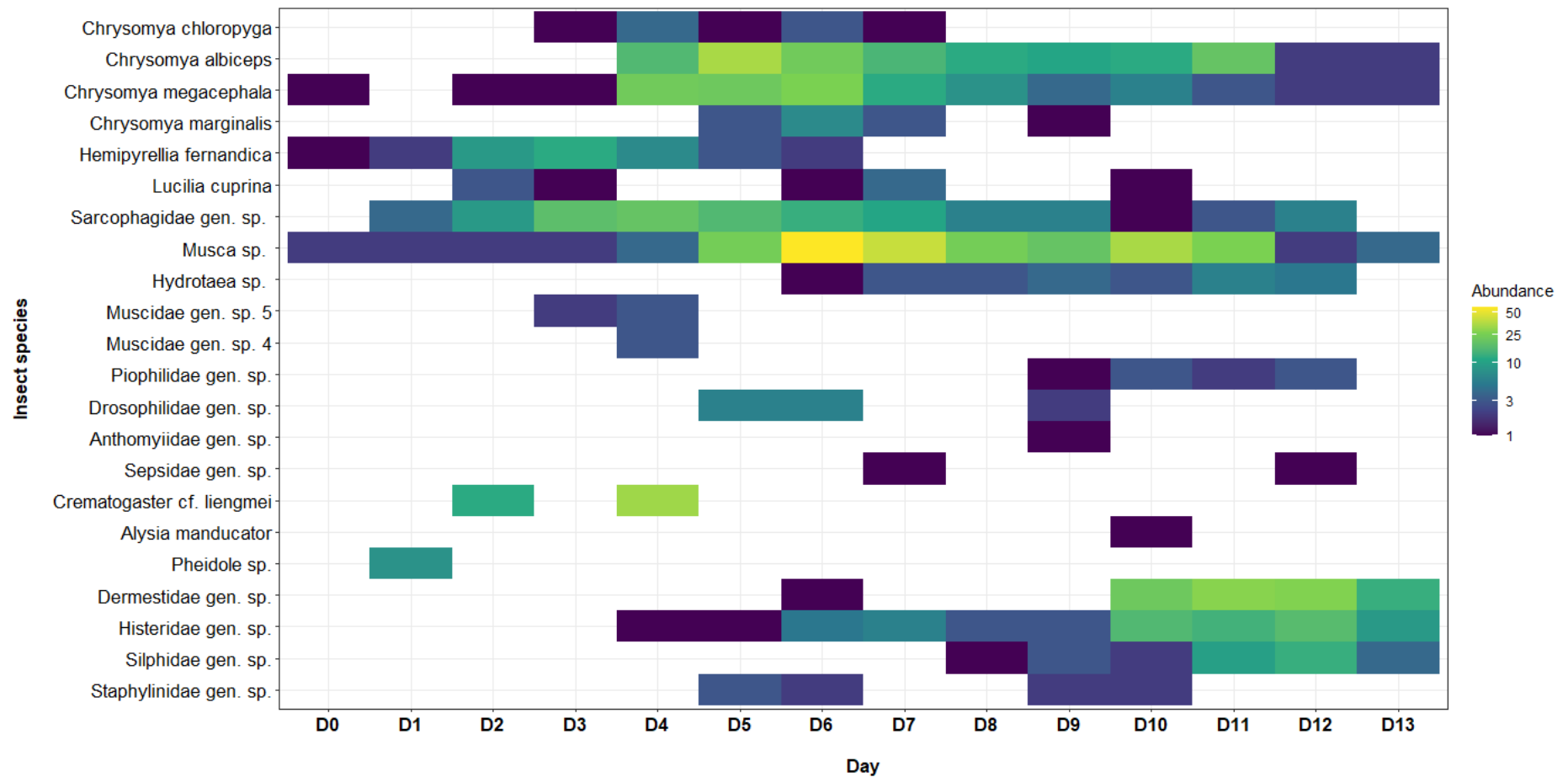


Figure B22: Daily abundance of insect species on pig carcass 2 in the late summer neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D6; Advanced decomposition: D7 – D9; Skeletonization: D10 – D13. L1 larvae of *Sarcophagidae gen. sp.* were recorded on Day 2; Eggs of *H. fernandica* and *Ch. megacephala* were recorded on Day 3 and 5 respectively.



Figure B23: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the mid-autumn neonate pig trial.



Figure B24: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the late autumn neonate pig trial.

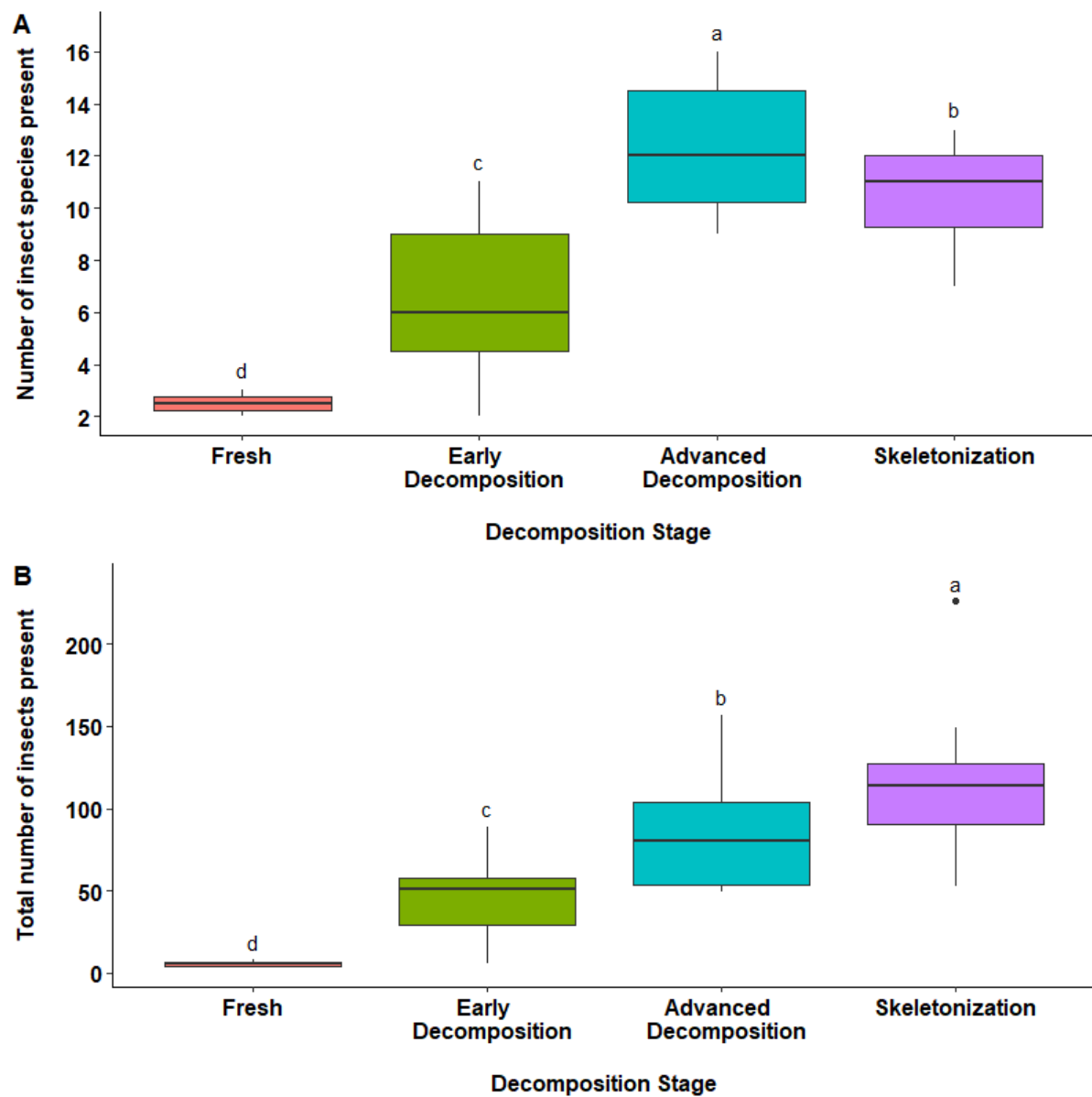


Figure B25: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the early autumn neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

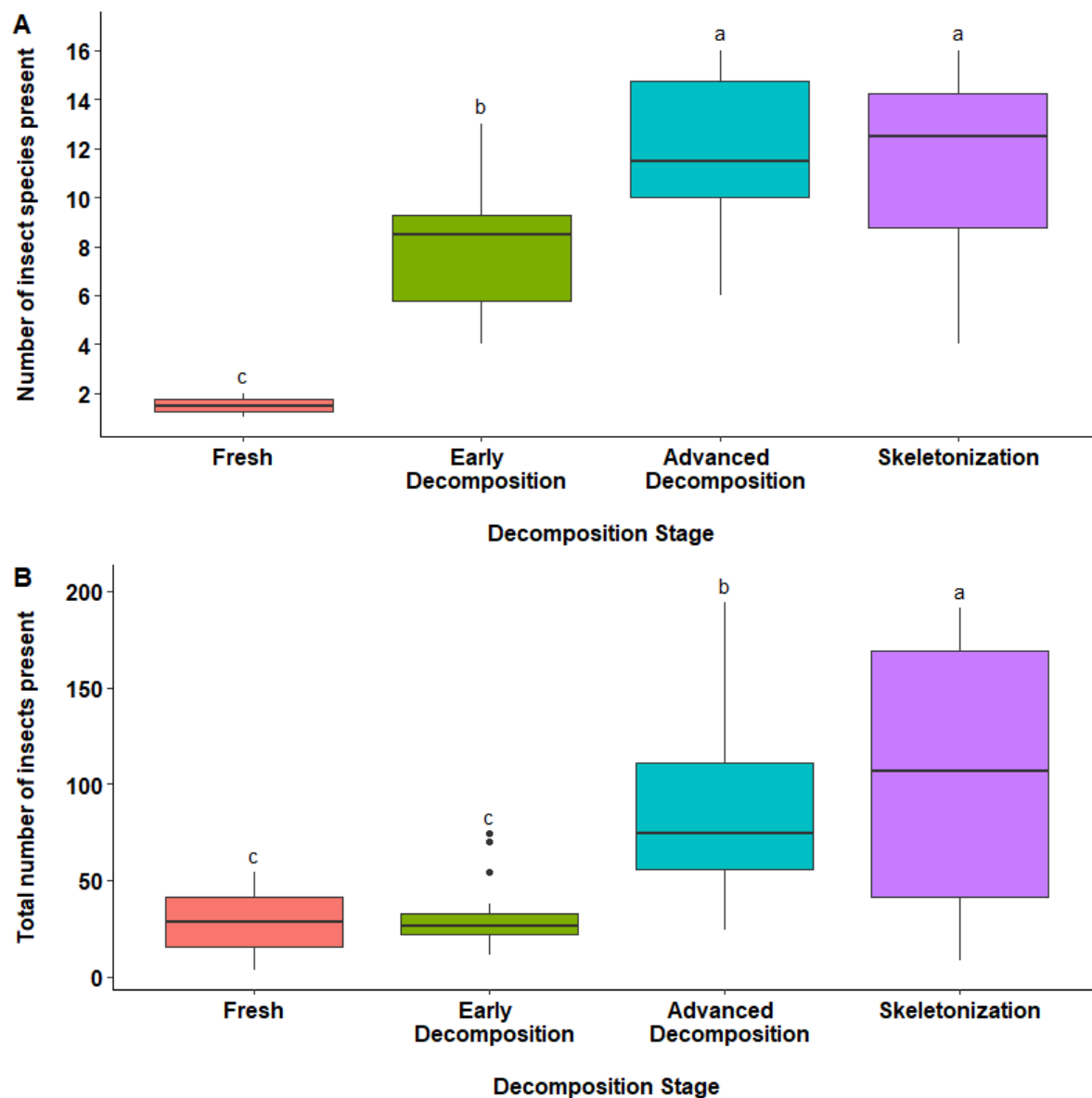


Figure B26: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the mid-autumn neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

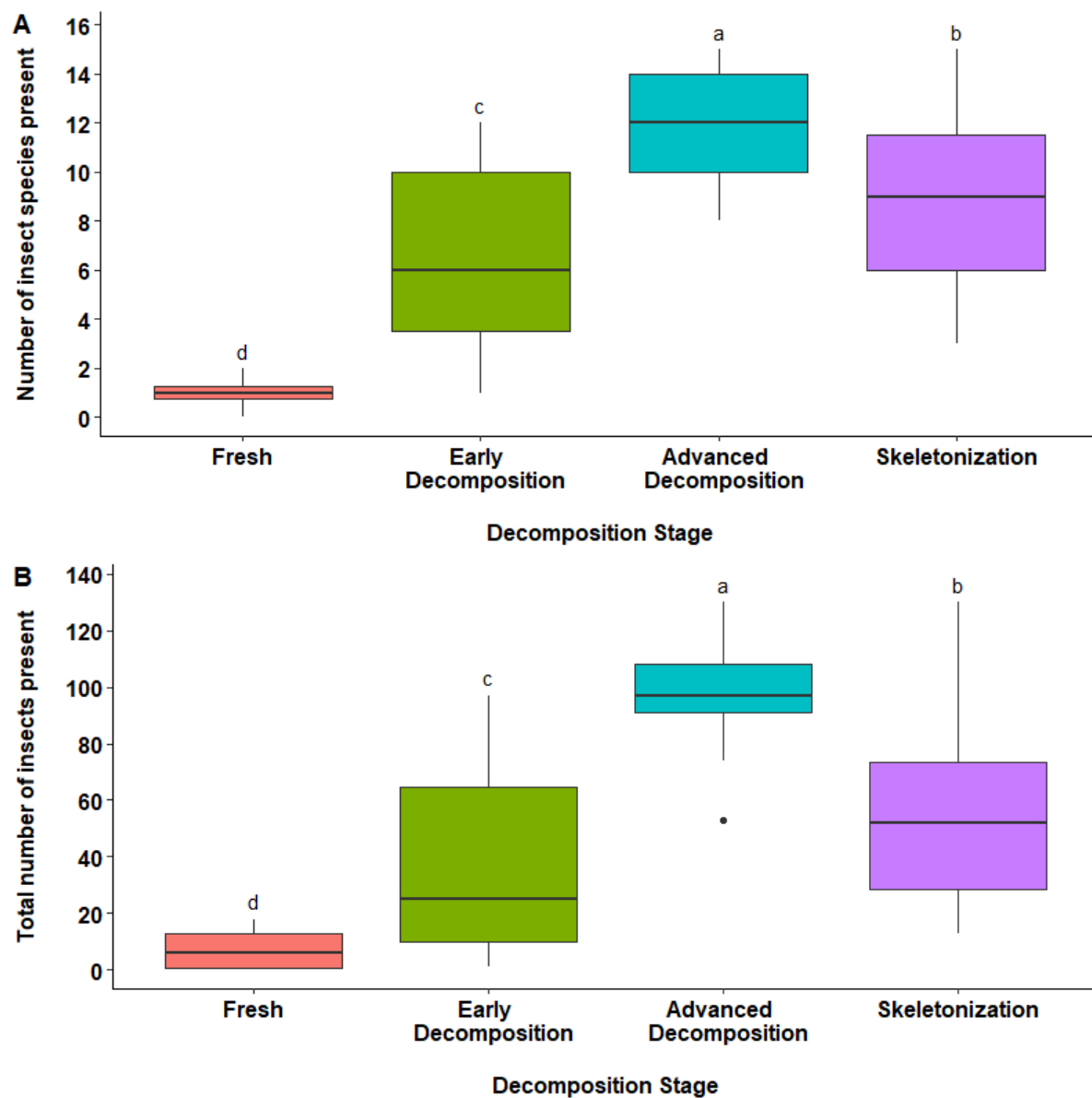


Figure B27: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the late autumn neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

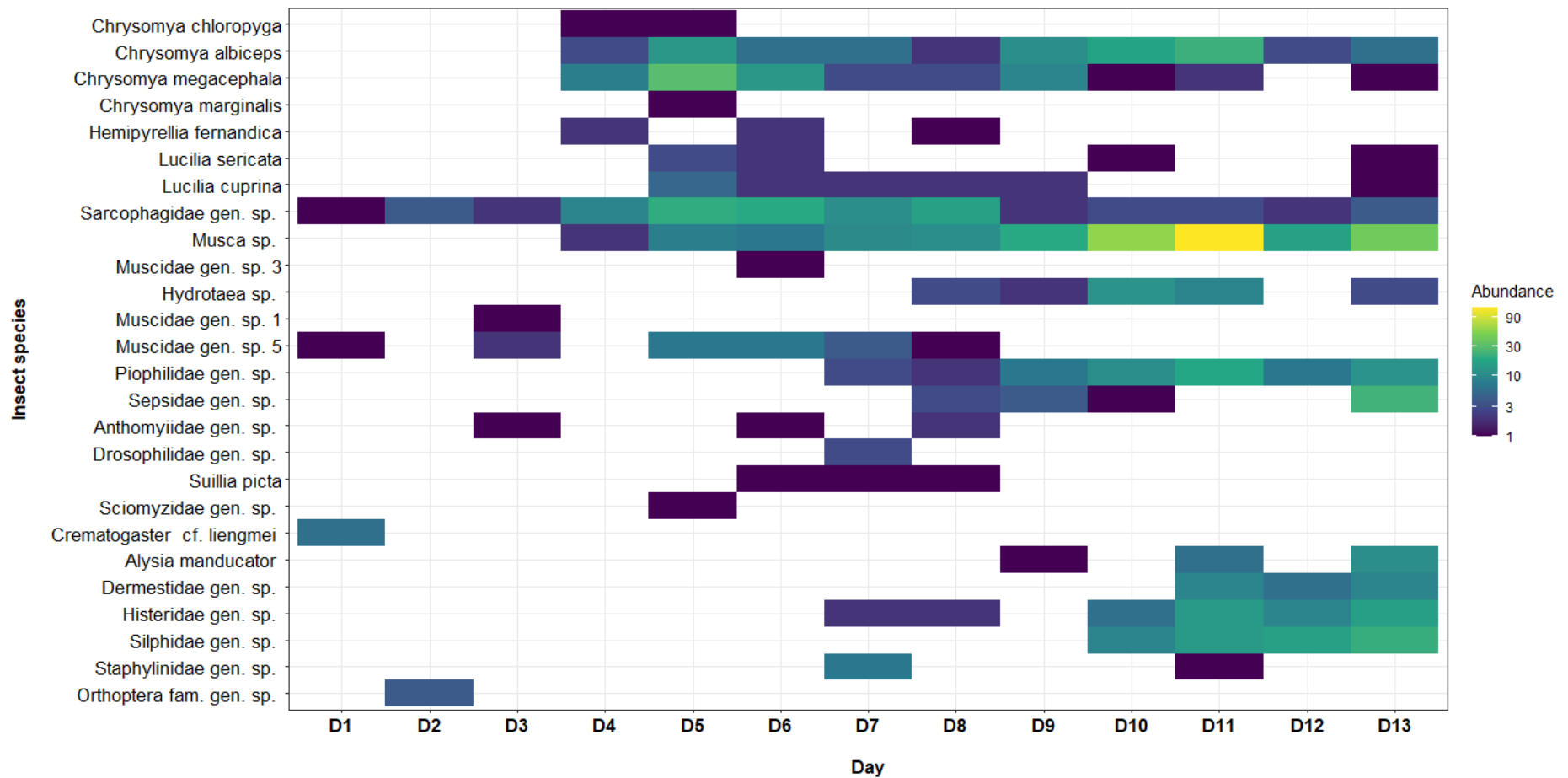


Figure B28: Daily abundance of insect species on pig carcass 1 in the early autumn neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D6; Advanced decomposition: D7 – D9; Skeletonization: D10 – D13. L1 and L2 larvae *Ch. chloropyga*, were recorded on Day 4; L1 and L2 larvae of *Sarcophagidae* gen. sp. were recorded on Day 4.

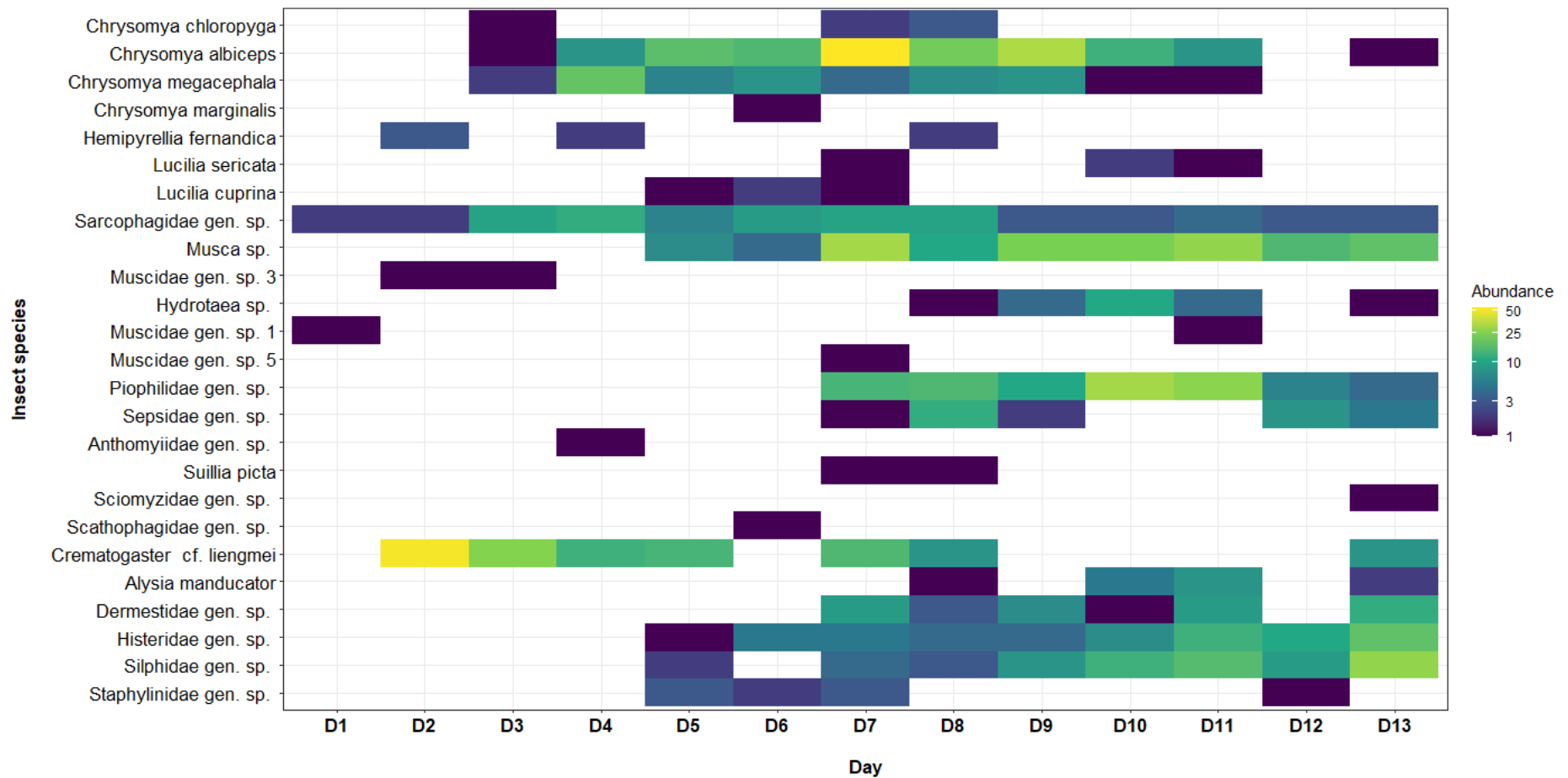


Figure B29: Daily abundance of insect species on pig carcass 2 in the early autumn neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D6; Advanced decomposition: D7 – D9; Skeletonization: D10 – D13. L1 and L2 larvae *Ch. chloropyga*, were recorded on Day 4.

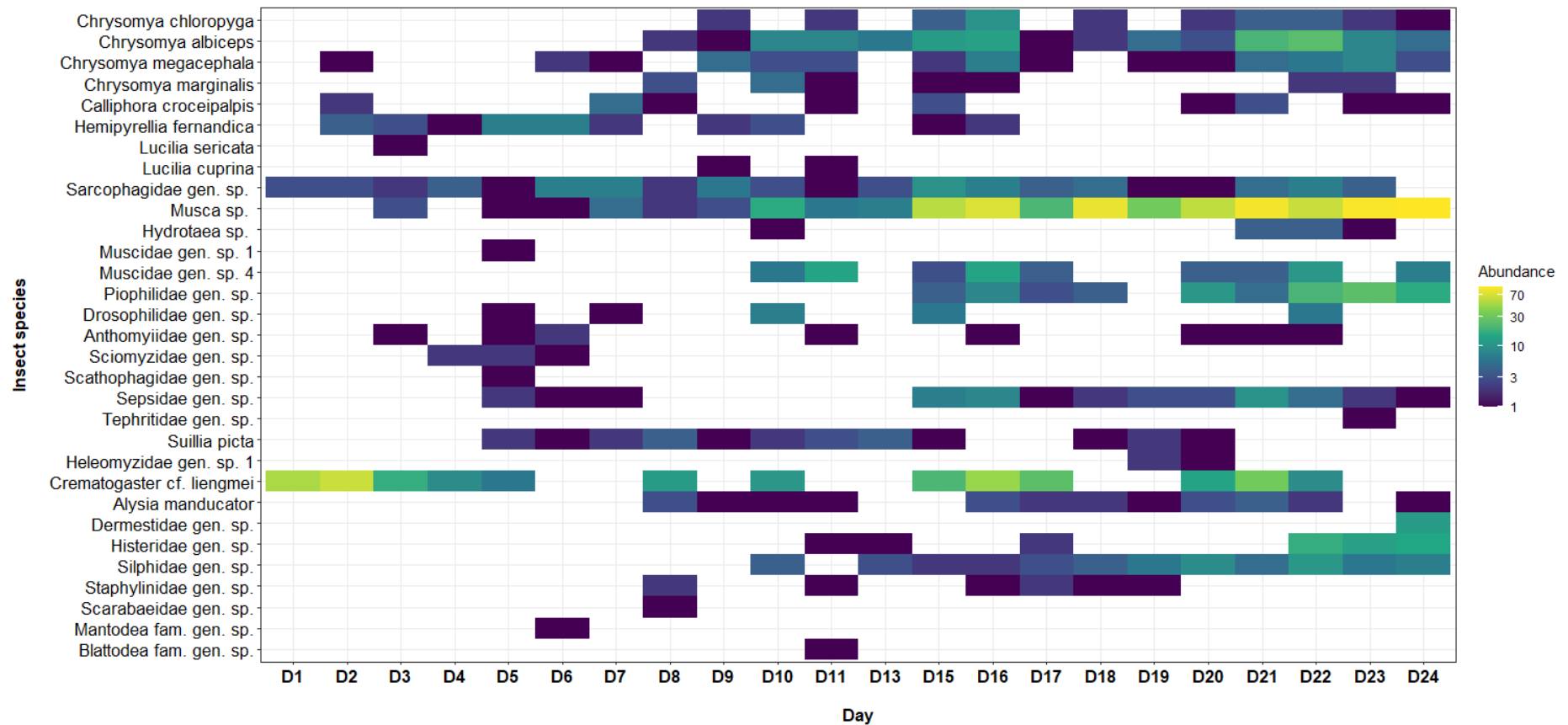


Figure B30: Daily abundance of insect species on pig carcass 1 in the mid-autumn neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D10; Advanced decomposition: D11 – D20; Skeletonization: D21 – D24. Eggs and L1 larvae of *H. fernandica* were recorded on Day 3; Eggs and L1 larvae of *Ch. chloropyga* were recorded on Day 7 and 8 respectively; Larvae of *Silphidae* gen. sp. were recorded on Day 20 until the end of the trial.

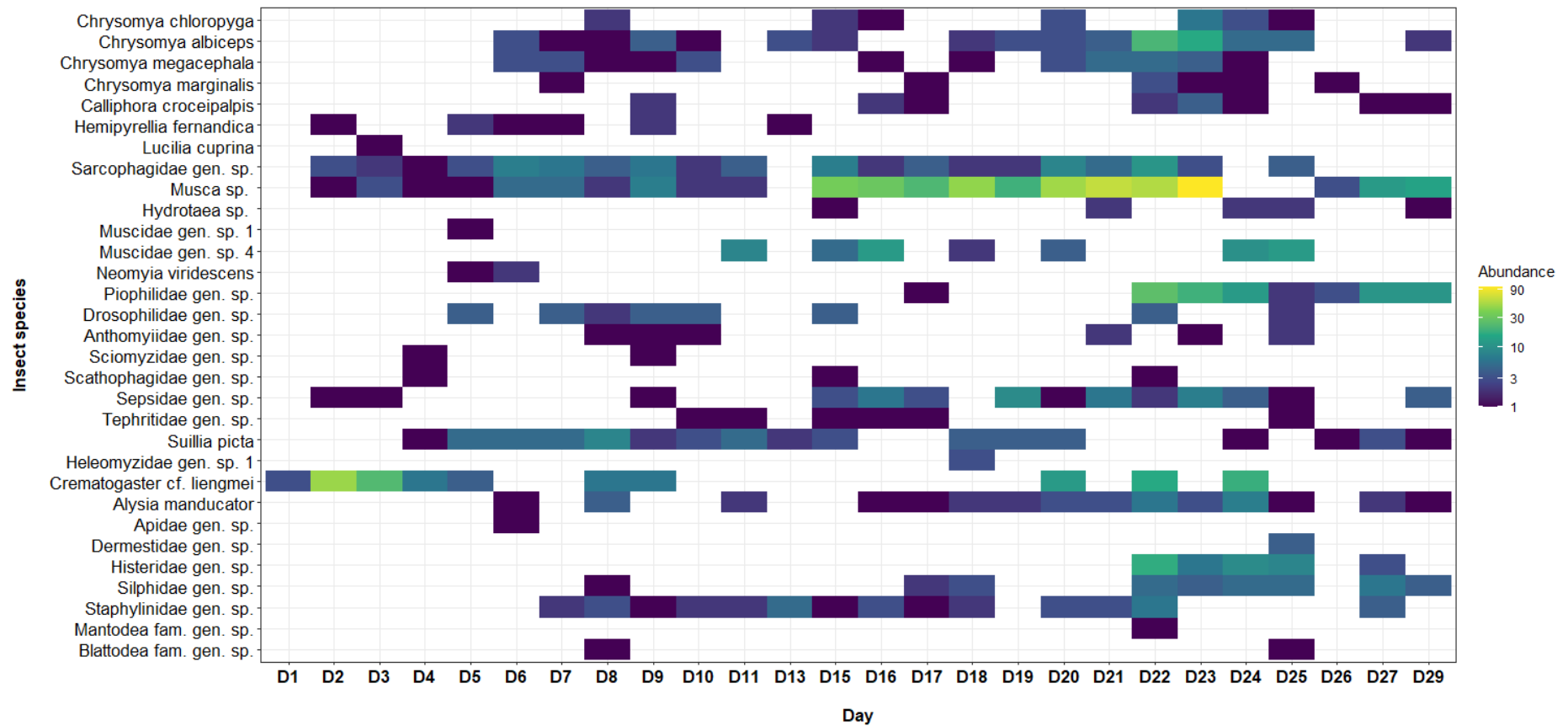


Figure B31: Daily abundance of insect species on pig carcass 2 in the mid-autumn neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D14; Advanced decomposition: D15 – D24; Skeletonization: D25 – D29. L1 larvae of *Sarcophagidae gen. sp.* were recorded on Day 3; Larvae of *Silphidae gen. sp.* were recorded on Day 29.

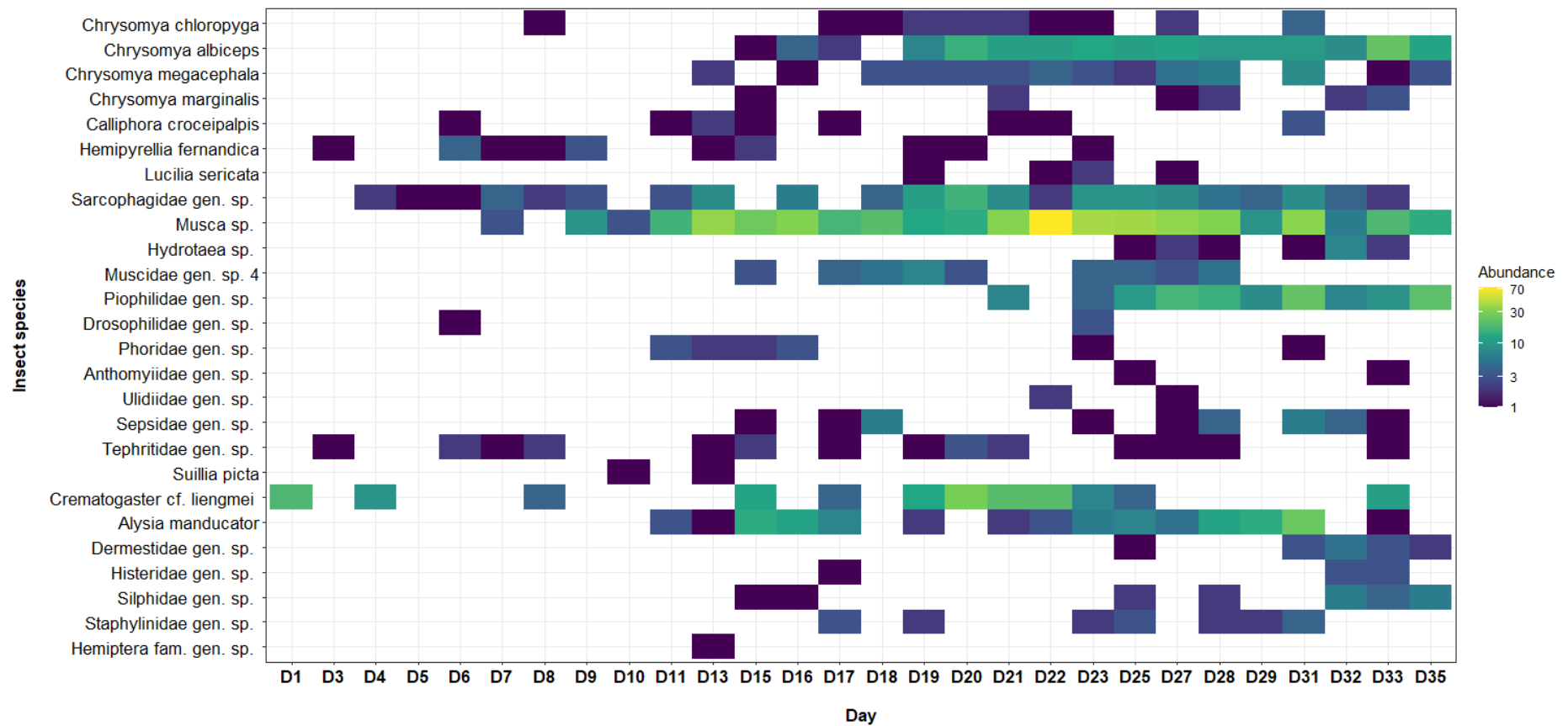


Figure B32: Daily abundance of insect species on pig carcass 1 in the late autumn neonate pig trial. Fresh stage: D0 – D2; Early decomposition: D3 – D21; Advanced decomposition: D22 – D28; Skeletonization: D29 – D35. Eggs and L1 larvae of *H. fernandica* were recorded on Day 6 and 7 respectively; Larvae of *Silphidae* gen. sp. were recorded on Day 31 until the end of the trial.

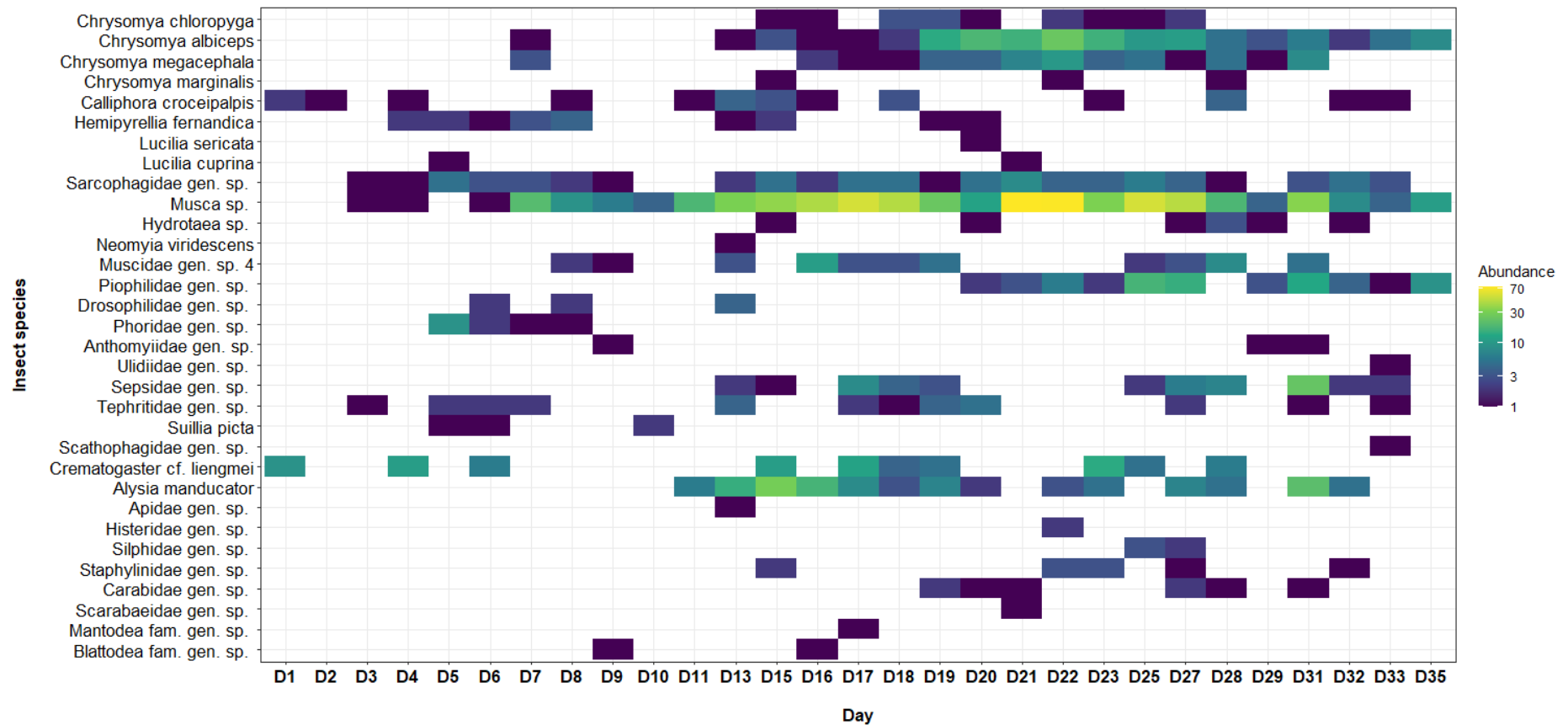


Figure B33: Daily abundance of insect species on pig carcass 2 in the late autumn neonate pig trial. Fresh stage: D0 – D2; Early decomposition: D3 – D18; Advanced decomposition: D19 – D27; Skeletonization: D28 – D35. Eggs and L1 larvae of *H. fernandica* were recorded on Day 2 and 3 respectively.



Figure B34: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the mid-winter neonate pig trial.



Figure B35: Photographs showing the decomposition stages (i.e., fresh, early decomposition, advanced decomposition and skeletonization respectively) of carcass 1 (A – D) and carcass 2 (E – H) during the late winter neonate pig trial.

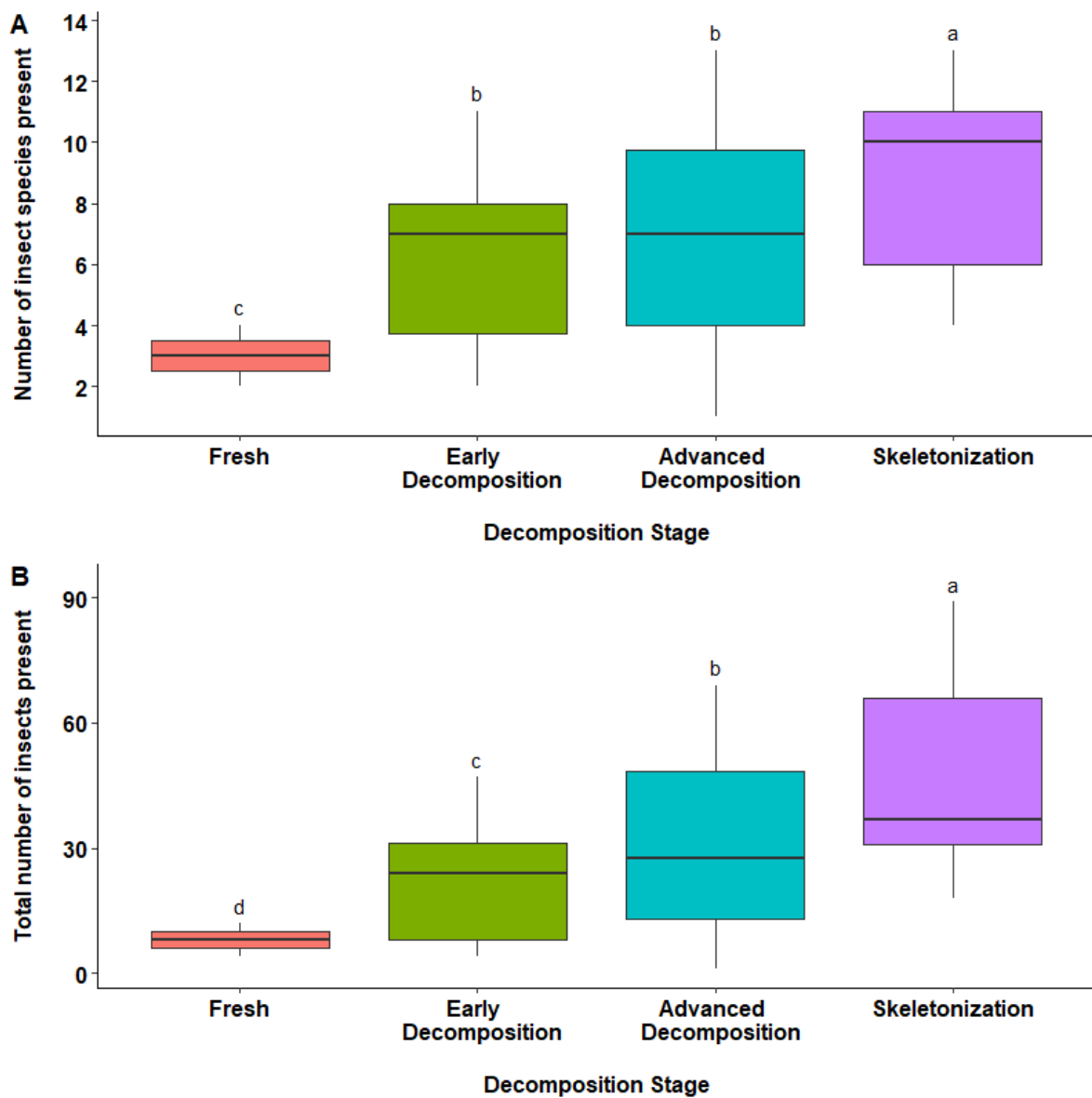


Figure B36: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the early winter neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

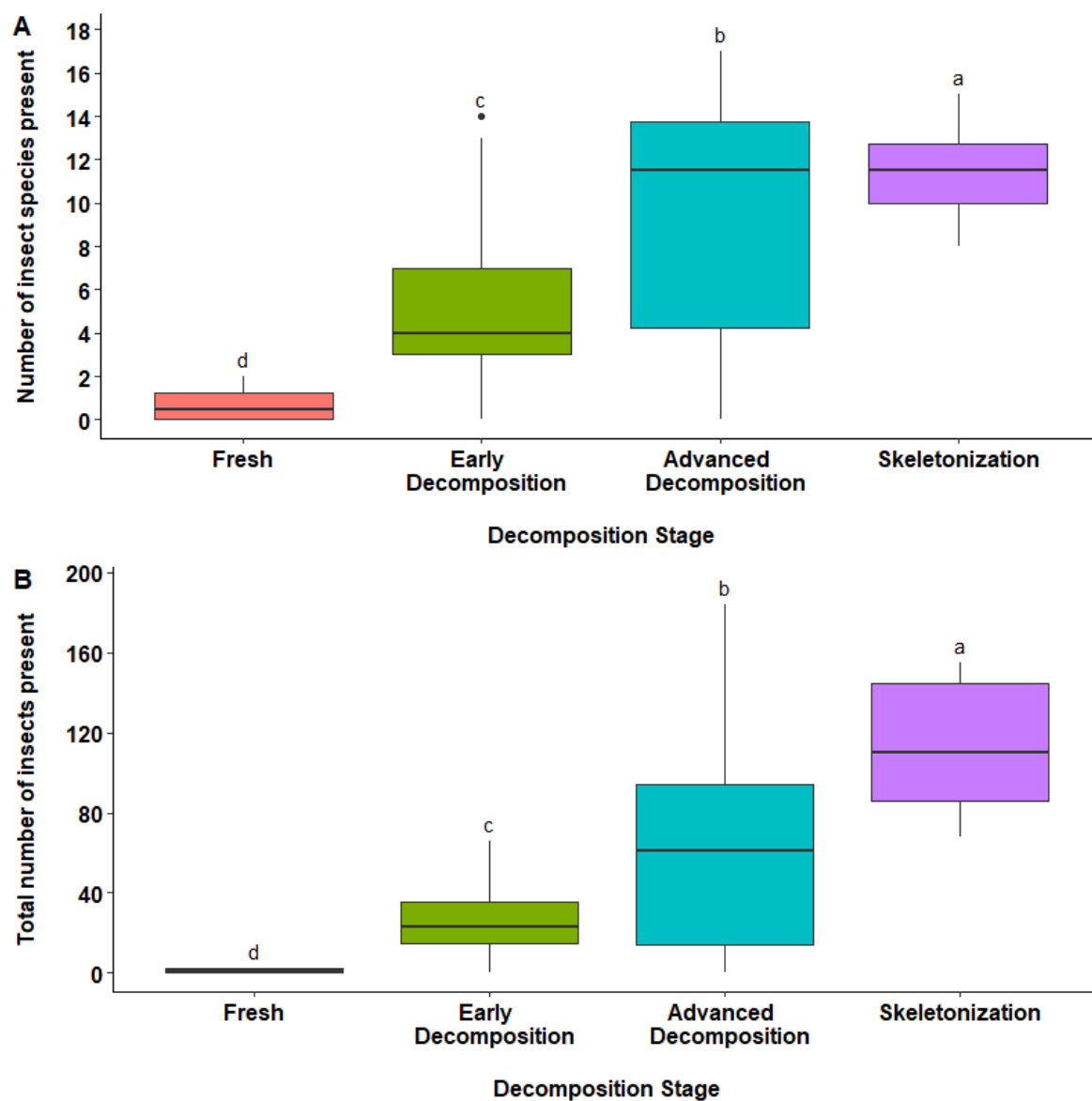


Figure B37: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the mid-winter neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

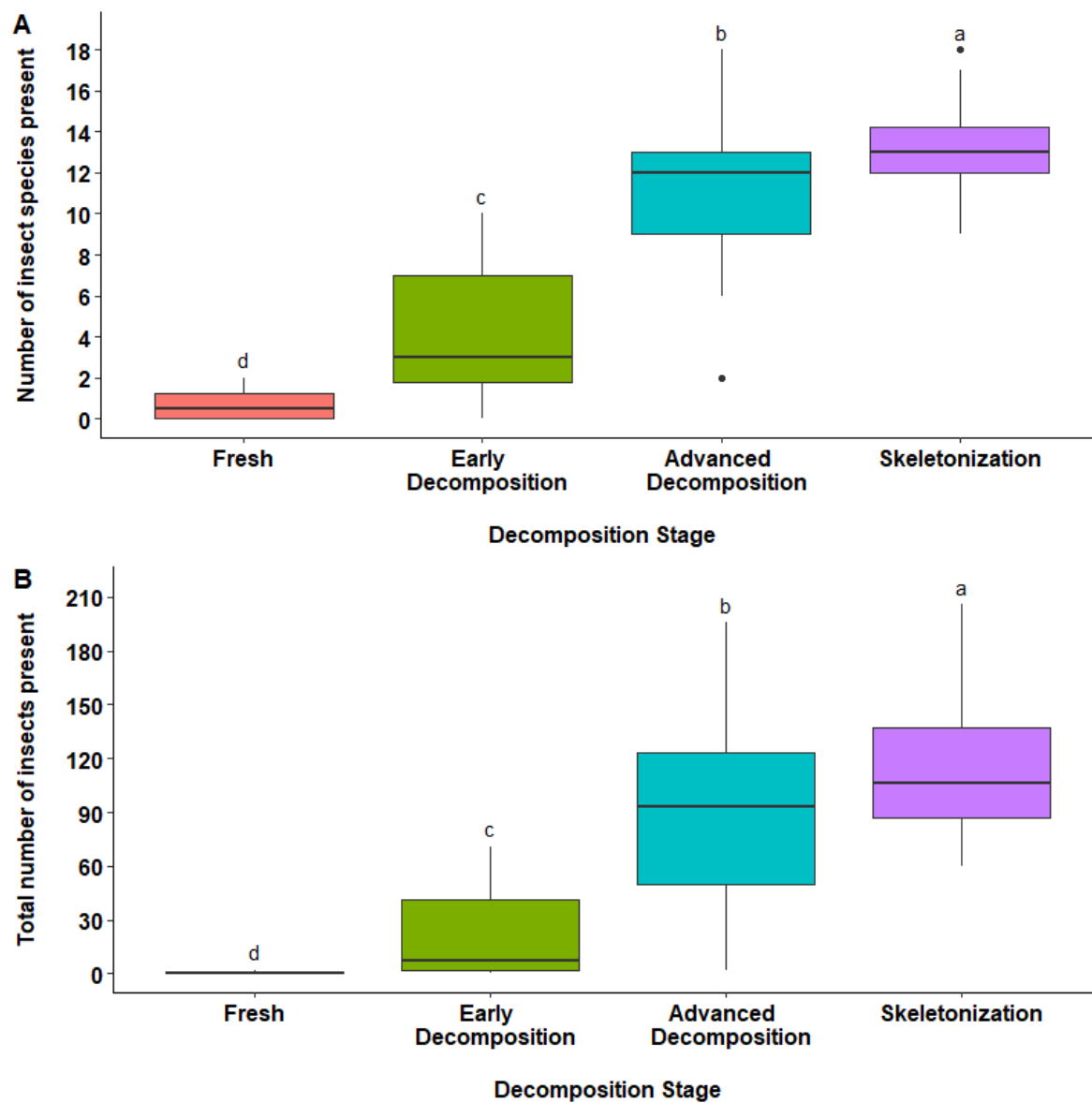


Figure B38: Box plots comparing the number of insect species (A) and total number of insects (B) present across the four decomposition stages in the late winter neonate pig trial. Boxes capped with different letters are significantly different after Tukey's HSD test ($p < 0.05$).

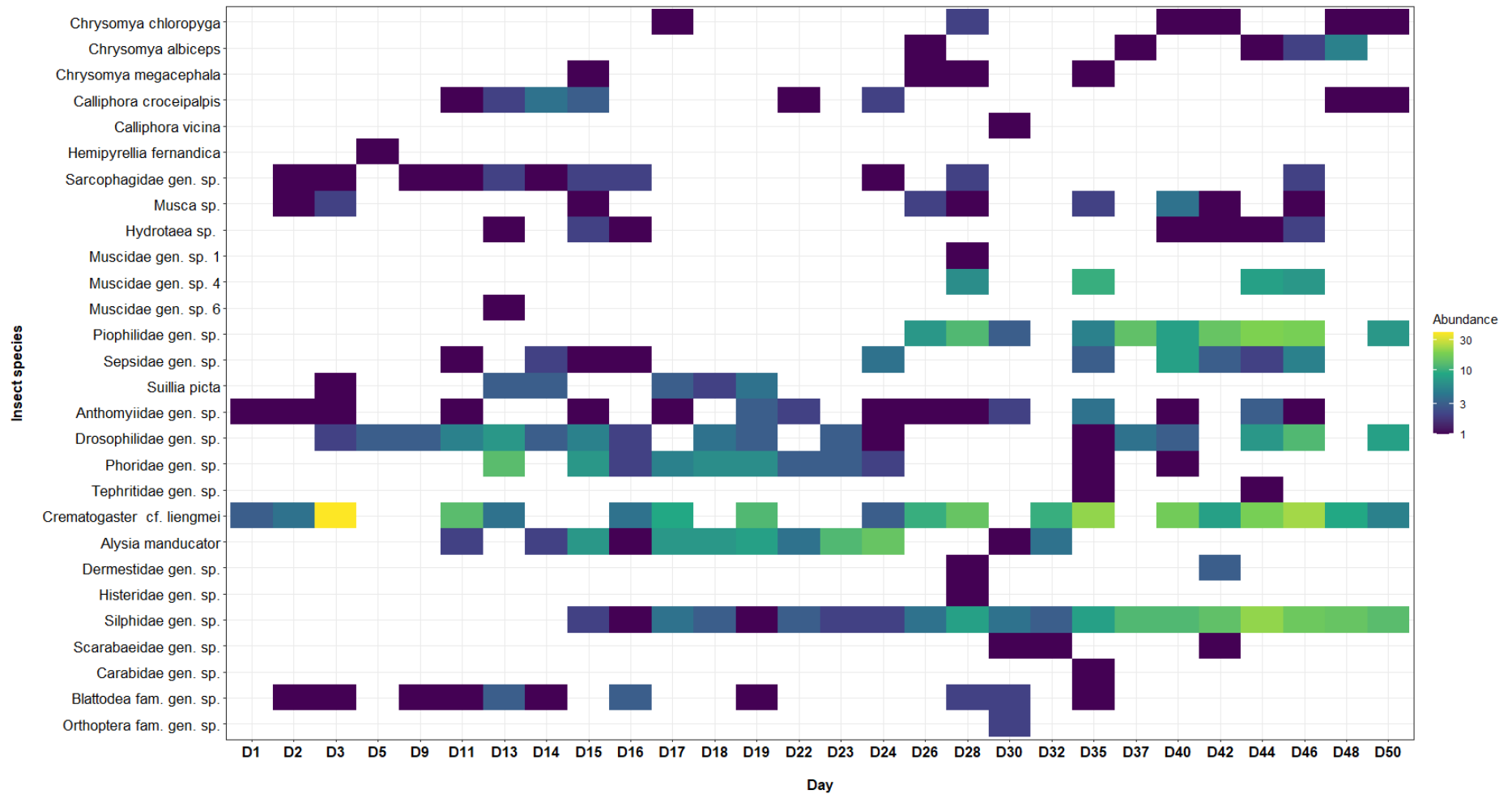


Figure B39: Daily abundance of insect species on pig carcass 1 in the early winter neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D21; Advanced decomposition: D22 – D43; Skeletonization: D44 – D50. The larvae of *Silphidae gen. sp.* were recorded on Day 32 until the end of the trial.

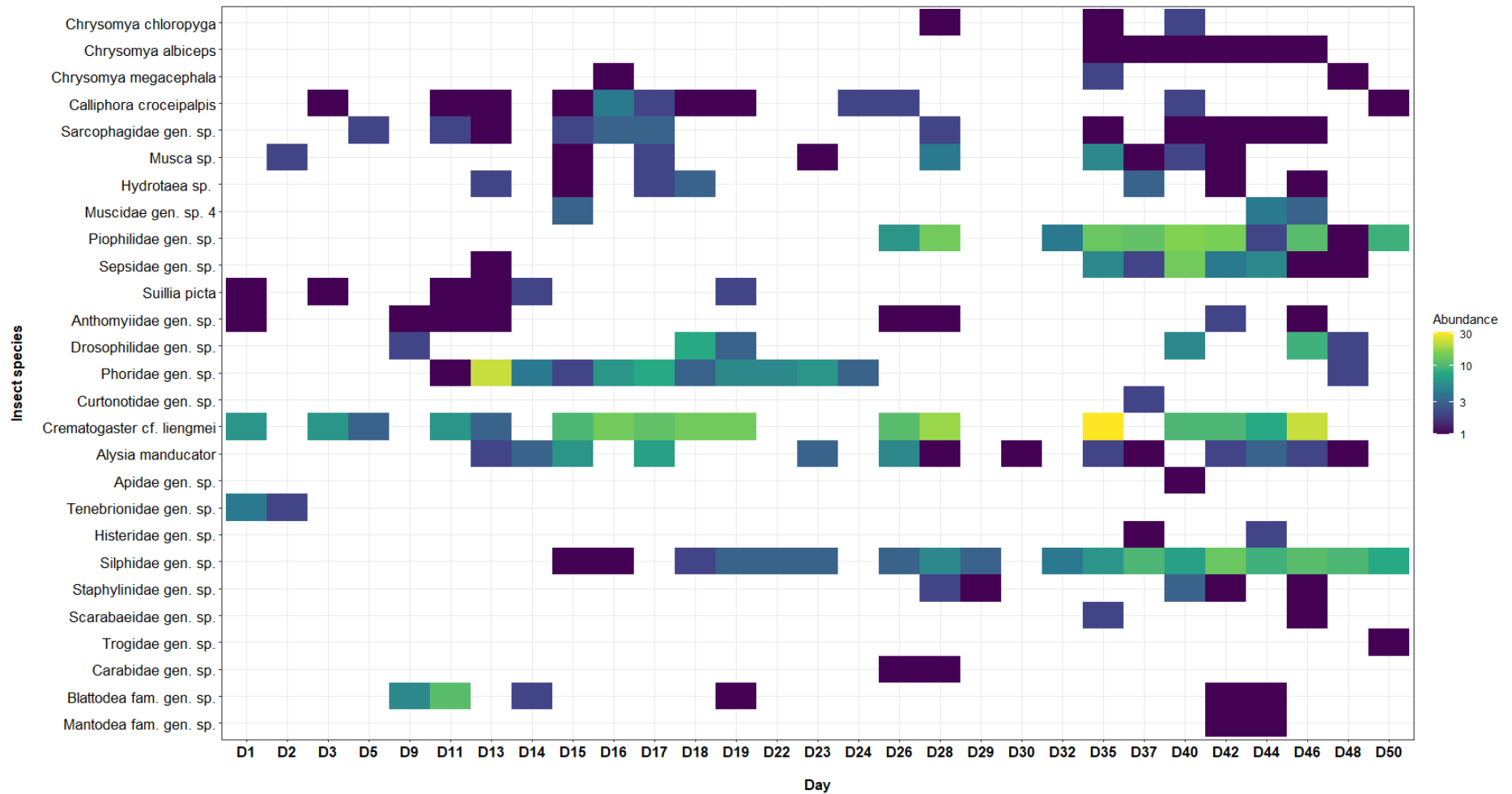


Figure B40: Daily abundance of insect species on pig carcass 2 in the early winter neonate pig trial. Fresh stage: D0 – D1; Early decomposition: D2 – D21; Advanced decomposition: D22 – D41; Skeletonization: D42 – D50. Eggs of *Ca. vicina* were recorded on Day 1; Eggs of *Ca. croceipalpis* were recorded on Day 11; Larvae of Silphidae gen. sp. were recorded on Day 40 until the end of the trial.



Figure B41: Daily abundance of insect species on pig carcass 1 in the mid-winter neonate pig trial. Fresh stage: D0 – D2; Early decomposition: D3 – D35; Advanced decomposition: D36 – D56; Skeletonization: D57 – D66. L1 larvae of Sarcophagidae gen. sp. were recorded on Day 32; Eggs and L1 larvae of *Ch. chloropyga*, were recorded on Day 32 and 34 respectively; Eggs of *Ca. vicina* were recorded on Day 38; Larvae of Silphidae gen. sp. were recorded on Day 36 until the end of the trial.



Figure B42: Daily abundance of insect species on pig carcass 2 in the mid-winter neonate pig trial. Fresh stage: D0 – D2; Early decomposition: D3 – D35; Advanced decomposition: D36 – D56; Skeletonization: D57 – D66; Larvae of Silphidae gen. sp. were recorded on Day 36 until the end of the trial.



Figure B43: Daily abundance of insect species on pig carcass 1 in the late winter neonate pig trial. Fresh stage: D0 – D2; Early decomposition: D3 – D17; Advanced decomposition: D18 – D40; Skeletonization: D41 – D48. Eggs and L1 larvae of *Ca. croceipalpis* were recorded on Day 3 and 8 respectively.



Figure B44: Daily abundance of insect species on pig carcass 2 in the late winter neonate pig trial. Fresh stage: D0 – D2; Early decomposition: D3 – D18; Advanced decomposition: D19 – D34; Skeletonization: D35 – D48. Eggs and L1 larvae of *Ca. croceipalpis* were recorded on Day 7 and 9 respectively; Eggs and L1/L2 larvae *Ch. chloropyga* were recorded on Day 25 and 27 respectively.

Appendix C: Supplementary Videos

Supplementary Video C1: Migration of blow fly larvae in the direction of the decomposition fluids (<https://tinyurl.com/42f7zcsv>).

Supplementary Video C2: *Crematogaster* cf. *liengmei* carrying fly eggs away from the carcass (<https://tinyurl.com/4kmf2efz>).

Supplementary Video C3: *Crematogaster* cf. *liengmei* individuals preying of a fly larva (<https://tinyurl.com/5aptz2v8>).

Supplementary Video C4: *Crematogaster* cf. *liengmei* individuals scavenging on an adult fly (<https://tinyurl.com/47h7z5p8>).

Appendix D: Life stages of insect species and families collected from decomposing neonate and adult pig carcasses and human remains in the Western Cape Province

Table D1: Sex, date of discovery, and insect species collected from 14 human forensic cases within the Western Cape Province of South Africa from 2015 to 2022.

Case	Decedent's sex	Month and Year	Season	Insect species collected	Collector
1	Female	October 2015	Spring	<i>L. sericata</i> (L) + Histeridae gen. sp. (A)	Forensic entomologist
2	Female	March 2016	Autumn	<i>Ch. chloropyga</i> (L and P) + <i>Ch. albiceps</i> (L) + <i>Ch. marginalis</i> (L) + Sarcophagidae gen. sp. (L) + Histeridae gen. sp. (A) + Dermestidae gen. sp. (A)	Pathologist
3	Male	August 2016	Winter	<i>Ca. vicina</i> (L) + <i>M. prolapsa</i> (L)	Pathologist
4	Female	October 2016	Spring	<i>Ch. chloropyga</i> (L and A) + <i>Ch. albiceps</i> (A) + <i>Lucilia</i> spp. (A) + <i>M. domestica</i> (A) + <i>T. mutilatus</i> (A) + Sepsidae gen. sp. (-)	Forensic entomologist
5	Male	December 2016	Summer	<i>Ch. albiceps</i> (L) + <i>Ch. marginalis</i> (L)	Pathologist
6	Male	November 2016	Spring	<i>Ch. albiceps</i> (L) + <i>Ch. marginalis</i> (L) + <i>T. mutilatus</i> (A) + <i>N. rufipes</i> (A)	Pathologist
7	Male	August 2016	Winter	<i>Ch. chloropyga</i> (L and P) + <i>Ch. albiceps</i> (P) + Piophilidae gen. sp. (L and P) + Staphylinidae gen. sp. (A)	Forensic entomologist

8	Male	January 2017	Summer	<i>Ch. marginalis</i> (P)	Forensic entomologist
9	Female	May 2017	Autumn	<i>Ch. marginalis</i> (P and Pc) + <i>Ch. albiceps</i> (P and Pc) + <i>H. capensis</i> (L) + <i>D. maculatus</i> (A) + <i>N. rufipes</i> (A)	Forensic entomologist
10	Female	February 2018	Summer	<i>Ch. albiceps</i> (L and A) + <i>Ch. marginalis</i> (A) + <i>L. cuprina</i> (L and A) + Sarcophagidae gen. sp. (A)	Forensic entomologist
11	Male	December 2021	Summer	<i>H. fernandica</i> (L) + <i>L. cuprina</i> (L) + <i>L. sericata</i> (L) + <i>Ch. albiceps</i> (L)	Pathologist
12	Male	January 2022	Summer	<i>Ch. megacephala</i> (L) + <i>Ch. marginalis</i> (L) + <i>Ch. albiceps</i> (L) + <i>Ch. chloropyga</i> (L) + Cleridae gen. sp. (A)	Pathologist
13	Male	January 2022	Summer	<i>Ch. chloropyga</i> (L) + <i>Ch. albiceps</i> (L)	Pathologist
14	Female	February 2022	Summer	Calliphoridae gen. sp. (P) + Piophilidae gen. sp. (L) + Dermestidae gen. sp. (L and A) + Cleridae gen. sp. (A) + Histeridae gen. sp. (A)	Forensic entomologist

Note: A = Adult stage; L = Larva stage; P = Pupa stage; Pc = Pupal casing; - = Unknown

Table D2: List of insect species collected from decomposing neonate and adult pig carcasses and human remains in the Western Cape Province.

Neonate pig carcasses	Adult pig carcasses	Human cadavers
<i>Ch. chloropyga</i> ^{Sp, Su, Au, Wi}	<i>Ch. chloropyga</i> ^{Su, Wi}	<i>Ch. chloropyga</i> ^{Au, Sp, Su, Wi}
<i>Ch. albiceps</i> ^{Su}	<i>Ch. albiceps</i> ^{Su}	<i>Ch. albiceps</i> ^{Au, Su, Sp, Wi}
<i>Ch. megacephala</i> ^{Su}	<i>Ch. megacephala</i>	<i>Ch. megacephala</i> ^{Su}
<i>Ch. marginalis</i> ^{Su}	<i>Ch. marginalis</i>	<i>Ch. marginalis</i> ^{Au, Su, Sp}
<i>Ca. vicina</i> ^{Sp, Wi}	<i>Ca. vicina</i>	<i>Ca. vicina</i> ^{Wi}
<i>Ca. croceipalpis</i> ^{Sp, Wi}	<i>Ca. croceipalpis</i>	<i>L. sericata</i> ^{Sp, Su}
<i>L. sericata</i>	<i>L. sericata</i>	<i>L. cuprina</i> ^{Su}
<i>L. cuprina</i>	<i>L. cuprina</i>	<i>Lucilia</i> spp.
<i>H. fernandica</i> ^{Su, Au}	<i>H. fernandica</i>	<i>H. fernandica</i> ^{Su}
<i>Pericallimyia</i> sp.	<i>Musca</i> sp.	Calliphoridae gen. sp. ^{Su}
<i>Musca</i> sp.	<i>Hydrotaea</i> sp.	<i>M. domestica</i>
<i>Hydrotaea</i> sp.	Muscidae gen. sp. 4	<i>M. prolapsa</i> ^{Wi}
<i>N. viridescens</i>	<i>S. picta</i>	<i>H. capensis</i> ^{Au}
Muscidae gen. sp. 1	Piophilidae gen. sp.	Piophilidae gen. sp. ^{Wi, Su}
Muscidae gen. sp. 2	Sepsidae gen. sp.	Sepsidae gen. sp.
Muscidae gen. sp. 3	Phoridae gen. sp.	Sarcophagidae gen. sp. ^{Au}
Muscidae gen. sp. 4	Anthomyiidae gen. sp.	<i>D. maculatus</i>
Muscidae gen. sp. 5	Drosophilidae gen. sp.	Dermestidae gen. sp. ^{Su}
Muscidae gen. sp. 6	<i>Bibio</i> sp.	<i>N. rufipes</i>
<i>S. picta</i>	Sarcophagidae gen. sp.	Cleridae gen. sp.
Heleomyzidae gen. sp. 1	<i>Crematogaster</i> cf. <i>liengmei</i>	Histeridae gen. sp.
Piophilidae gen. sp.	<i>A. manducator</i>	Staphylinidae gen. sp.
Sepsidae gen. sp.	Vespidae gen. sp.	<i>T. mutilatus</i>
Phoridae gen. sp.	Tenebrionidae gen. sp.	
Anthomyiidae gen. sp.	Cleridae gen. sp.	
Drosophilidae gen. sp.	Trogidae gen. sp.	
Sciaridae gen. sp.	Scarabaeidae gen. sp.	
Curtonotidae gen. sp.	Silphidae gen. sp. ^{Wi}	

Scathophagidae gen. sp.	Dermestidae gen. sp. ^{Su, Wi}
Tephritidae gen. sp.	Histeridae gen. sp.
Sciomyzidae gen. sp.	Staphylinidae gen. sp.
Lauxanidae gen. sp.	Hemiptera fam. gen. sp.
Ulidiidae gen. sp.	Blattodea fam. gen. sp.
Sarcophagidae gen. sp. ^{Sp, Su, Au, Wi}	
<i>Crematogaster</i> cf. <i>liengmei</i>	
<i>Pheidole</i> sp.	
<i>Lepisiota</i> sp.	
<i>A. manducator</i>	
Apidae gen. sp.	
Vespidae gen. sp.	
Tenebrionidae gen. sp.	
Cleridae gen. sp.	
Trogidae gen. sp.	
Scarabaeidae gen. sp.	
Silphidae gen. sp. ^{Sp, Au, Wi}	
Dermestidae gen. sp. ^{Sp}	
Histeridae gen. sp.	
Staphylinidae gen. sp.	
Carabidae gen. sp.	
Coccinellidae gen. sp.	
Hemiptera fam. gen. sp.	
Blattodea fam. gen. sp.	
Orthoptera fam. gen. sp.	
Mantodea fam. gen. sp.	
Odonata fam. gen. sp.	

Note: Superscripts (Sp = Spring; Su = Summer; Au = Autumn; Wi = Winter) indicate the season in which the immature stages (eggs, larvae, pupae, and/or pupal casings) of the insect species were collected from/on the pig/human remains and taken to the Laboratory for rearing and identification.

Appendix E: Changes in control soil pH and electrical conductivity data between the adult and neonate pig trials

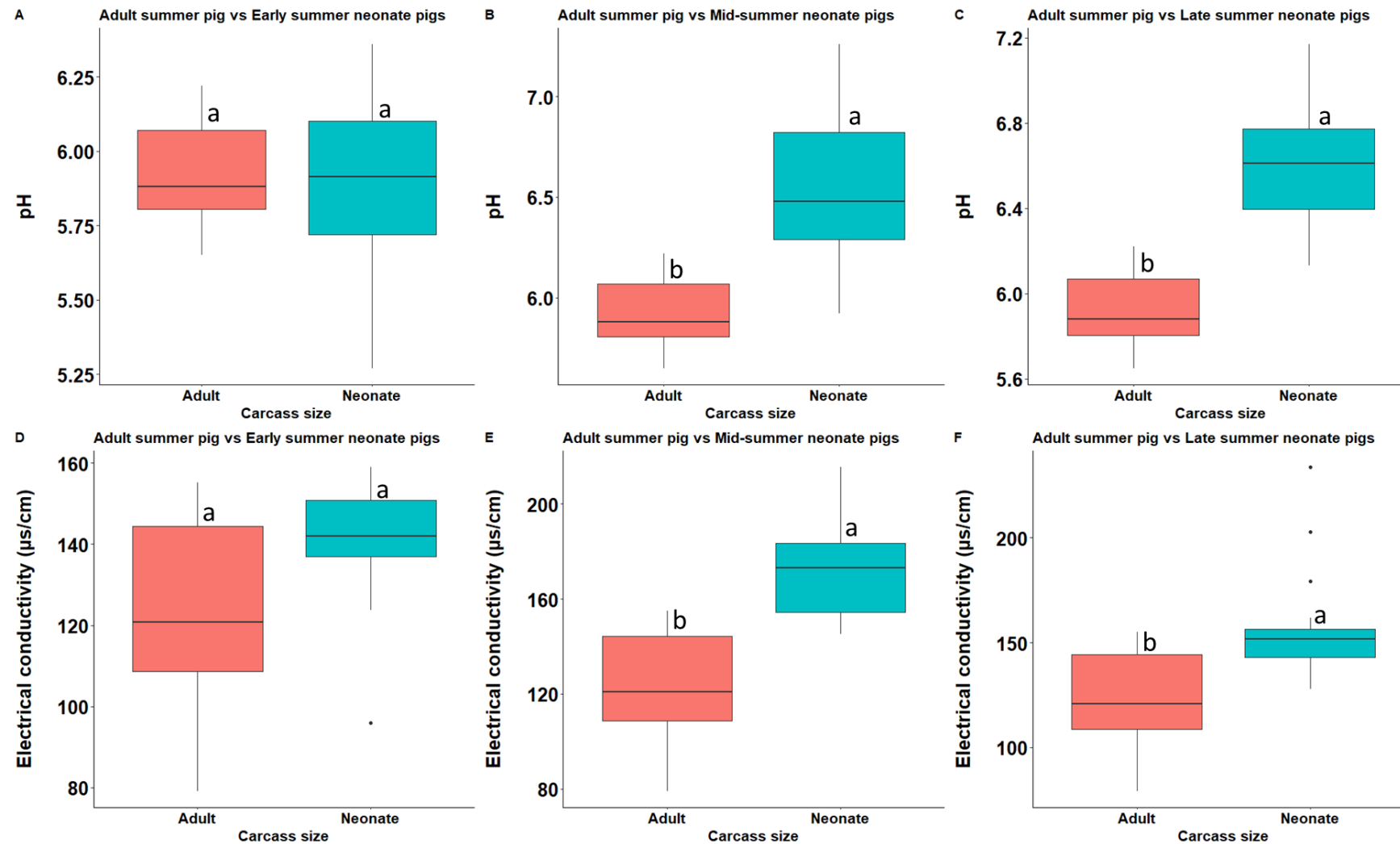


Figure E1: Box plots comparing the control soil pH (A – C) and electrical conductivity (D – E) of the adult summer pig carcass and neonate pig early, mid-, and late summer carcasses. Boxes capped with different letters are significantly different after Welch Two Sample t-test or Wilcoxon signed-rank test ($p < 0.05$).

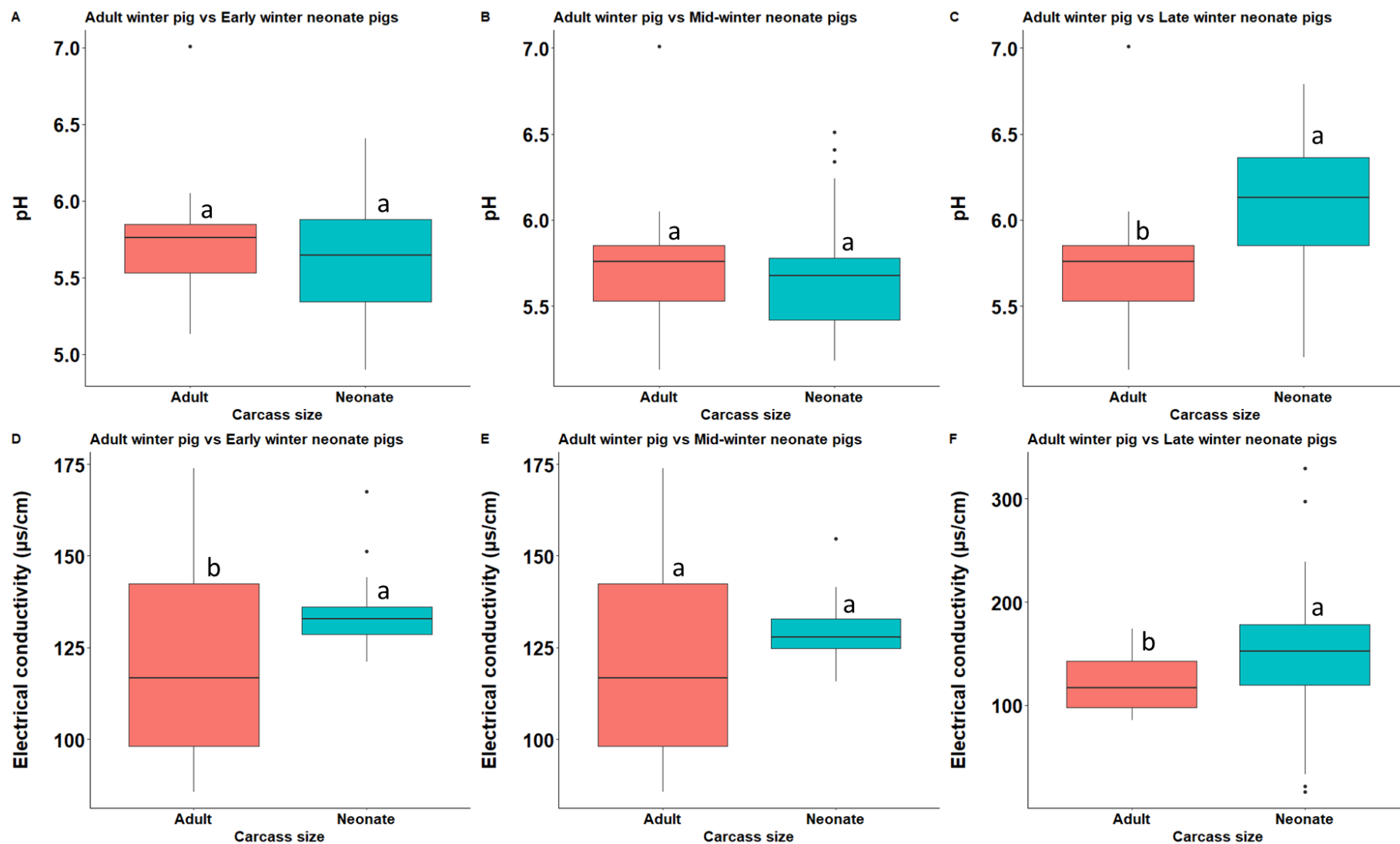


Figure E2: Box plots comparing the control soil pH (A – C) and electrical conductivity (D – E) of the adult winter pig carcass and neonate pig early, mid-, and late winter carcasses. Boxes capped with different letters are significantly different after Welch Two Sample t-test or Wilcoxon signed-rank test ($p < 0.05$).